GENETICS OF RESISTANCE TO POTYVIRUSES IMPACTING SNAP BEAN (*Phaseolus vulgaris* L.) PRODUCTION IN THE UNITED STATES

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Snap bean (*Phaseolus vulgaris* L.) is an important crop to diversified vegetable production in the Great Lakes Region of the United States, but an aphid-transmitted virus disease complex threatens sustainable production. The component viruses include the potyviruses *Clover yellow vein virus* (ClYVV) and *Bean yellow mosaic virus* (BYMV) that are acquired and transmitted rapidly by dispersing aphid vectors. This situation necessitates the identification, introgression, and deployment of plant virus resistance alleles to achieve effective and ecologically sensitive control.

Genetic variation for resistance to ClYVV was characterized at the phenotypic and molecular genetic levels. The relationship of three previously putative independent resistance alleles, *cyv*, *desc*, and *bc-3* was resolved into an allelic series at the *Bc-3* locus where the strain and species-specific resistance spectrum was allele specific. Given previous advances, this pathosystem presented an immediate candidate gene, *P. vulgaris eIF4E* (*PveIF4E*), as the molecular basis for resistance. A complete association between specific non-synonymous single nucleotide polymorphisms (SNPs) and virus resistance led to the identification of the putative molecular determinants for resistance to ClYVV and *Bean common mosaic necrosis virus* (BCMNV) strain NL 3 D. *PveIF4E* allele specific assays were developed for rapid introgression of *bc-3* and the novel *bc-3*^{2} allele into bean breeding programs.

Phenotypic evaluation and the allele specific assays were used to further characterize ClYVV resistance in a large and representative sample of common bean genetic diversity. The result was the identification of novel ClYVV resistance in all major market classes, and the validation of the use of the assays for allele mining in germplasm collections. These efforts also lead to the phenotypic identification of ClYVV resistance alleles at independent loci, and established a model for resistance that now includes *By-2* and the *bc-u, bc-2*^{2} combination.

The novel and highly efficient genotyping-by-sequencing (GBS) method was adapted to common bean and used to map the position of the *By-2* allele for resistance to BYMV and ClYVV. *By-2* was mapped to within a 974k kb region on the distal portion of chromosome 2. This effort generated genomic resources for fine mapping and assays that were also validated for marker-assisted selection.

BIOGRAPHICAL SKETCH

John Patrick Hart grew up in Moon Township, a suburb of Pittsburgh, Pennsylvania. He graduated from Moon Area High School in 1999, and completed a B.Sc. in Global Resource Systems and Agroecology from the University of British Columbia in 2004. He went on to develop a wide range of interests and capacities as Research Director and Inventory Manager at West Coast Seeds, a vegetable seed distributor in Delta, British Columbia. This is where he realized that as a plant breeder, his work could potentially have real impact in enhancing agroecosystems, biodiversity, food security, and the pleasure of eating. These are his overall goals, and this is what brought him to the Department of Plant Breeding and Genetics at Cornell University in 2008. He is happiest outdoors, in the fields, gardens, and forests, and in the kitchen with his wife, friends, and family.

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CHAPTER 1

TRANSLATIONAL GENETICS FOR RESISTANCE TO VIRUS DISEASES OF SNAP BEAN IN THE UNITED STATES

1.1 APHID-TRANSMITTED VIRUS DISEASE COMPLEX OF SNAP BEAN

An aphid-transmitted virus disease complex has emerged as the cause of substantial economic damage to snap bean (*Phaseolus vulgaris* L.) production and processing in the Great Lakes Region of the United States (Larsen et al., 2002; Larsen et al., 2008; Nault et al., 2004; Shah et al., 2006; Tolin and Langham, 2010). The increase in virus disease incidence and severity has been associated with the increasing prevalence of the soybean aphid (*Aphis glycines* Matsumura), an introduced and efficient vector of legume viruses (Gildow et al. 2008). While the sampling of affected snap bean fields has revealed that the predominant virus is *Cucumber mosaic virus* (CMV) (genus *Cucumovirus,* family *Bromoviridae*), *Bean yellow mosaic virus* (BYMV) (genus *Potyvirus,* family *Potyviridae*) and *Clover yellow vein virus* (ClYVV, family *Potyviridae*, genus *Potyvirus*) are also widespread (Shah et al., 2006). The emergence of an aphid-transmitted virus disease complex of snap beans is particularly threatening to this region because it is the premier region of production in the United States where snap beans can generate greater than $185 million in farm gate revenue per year (USDA-NASS, 2013).

Few options exist to attempt to control epidemics and crop loss caused by these nonpersistently transmitted viruses. The use of pesticides to attempt to control the aphid vectors of non-persistently transmitted viruses has proven ineffective and control must be preventative (Nault et al., 2004). The most effective, efficient, and

ecologically sensitive control strategy is to develop cultivars with resistance to the viruses. No snap bean cultivars are available with resistance to CMV, BYMV, or ClYVV infection alone, or to any combination of these viruses.

The goals of this research were to elucidate the genetics of resistance to ClYVV and BYMV and to develop tools for both applied marker-assisted selection as well as for further fundamental dissection of the biological mechanisms underlying virus resistance. The ultimate goal is that the research will be a meaningful and useful contribution to protecting and enhancing snap bean production in the Great Lakes Region of the United States. The proposed objectives that supported these goals were the following:

Objective 1: Investigate the potential role of eukaryotic translation initiation factor *eIF4E* in resistance to *Clover yellow vein virus* (ClYVV) in common bean.

Objective 2: Elucidate the molecular genetics of resistance to *Bean yellow mosaic virus* (BYMV) in common bean.

Objective 3: Determine the genomic position and identify molecular markers tightly linked to the *bc-3* and *By-2* alleles that condition resistance to ClYVV, and BYMV and ClYVV respectively in common bean.

1.2 PREVALENT VIRUSES AND GENETIC RESISTANCE

1.2.1 *Clover yellow vein virus* (ClYVV)

Clover yellow vein virus (ClYVV) (genus *Potyvirus*, family *Potyviridae*) particles are long and filamentous, and contain a single stranded RNA that is approximately 9.5kb (ICTVdb, 2006a). The symptoms caused by ClYVV include a

wide range of leaf and whole plant symptoms, but also often include a high frequency of mottled, twisted, and malformed pods as well as interior pod necrosis referred to as 'chocolate pod' (Larsen et al. 2008). Snap beans are produced for fresh market use, freezing, or canning, with malformed and necrotic pods being rejected and discarded by wholesalers and processors. In some cases this has resulted in total yield loss for specific production fields and producers. Control measures including the use of pesticides to control the aphid vectors of ClYVV are generally ineffective because the virus is transmitted in a nonpersistent manner. The most effective, efficient, and ecologically sensitive control strategy is to develop cultivars with resistance to the virus.

Resistance to ClYVV in common bean has been identified and reported in the literature. In all reported cases, resistance to ClYVV is inherited recessively as a single recessive gene. The *cyv* resistance allele was identified in the great northern bean line US1140 (Provvidenti and Schroeder, 1973) and was transferred to the black bean cultivar Black Knight (Scully et al., 1995). The *cyv* allele was also identified in the black bean cultivar Kentwood having been inherited from the navy bean cultivar Clipper (Tu, 1983). A single recessive gene was also identified in by Sato et al. (2003) in the cultivar Jolanda, and designated *desc*, but there appears to be a lack of evidence as to whether or not this was a novel gene, or whether it was the *cyv* resistance gene, or possibly the *bc-3* resistance gene described below.

The *bc-3* virus resistance gene is one of four loci that condition resistance to the important potyviruses *Bean common mosaic virus* (BCMV) (genus *Potyvirus*, family *Potyviridae*) and *Bean common mosaic necrosis virus* (BCMNV) (genus

Potyvirus, family *Potyviridae*). The *bc-3* resistance gene has also recently been confirmed to condition complete resistance to ClYVV (Larsen et al., 2008) and was reported as tightly linked and non-allelic to the *cyv* resistance gene (Larsen, 2006). Given that the susceptibility of plants to viruses relies on factors provided by the host, recessive resistance to viruses, particularly those species of the family *Potyviridae* is quite common (Fraser, 1990; Kang et al., 2005; and Robaglia and Caranta, 2006). Decades of research advances in understanding the genetic and functional basis for recessive resistance to the family *Potyviridae* has lead to the conclusion that host translation initiation factors play a central role in the successful infection of plants to potyviruses (Kang et al., 2005; and Robaglia and Caranta, 2006).

Resistance to potyviruses has been confirmed when mutations in host translation initiation factors create nonfunctional recessive resistance alleles. Naturally occurring mutations in members of the *eIF4E* and *eIF4G* families of host translation initiation factors have been demonstrated to be responsible for recessive resistance to viruses in many if not most important crop species (Kang et al., 2005; Robaglia and Caranta, 2006). The information generated by these studies has characterized in great detail the genes, functional mutations, and plant-virus interactions responsible for the phenomena of recessive virus resistance.

This characterization, coupled by the conservation of DNA and amino acid sequence of host translation initiation factors across crop-plant families and species has lead to the ability to search for naturally occurring mutations in these factors in other species. The hypothesis that a mutated *eIF4E* allele may be the underlying factor of resistance to ClYVV in common bean was therefore examined.

1.2.2 *Bean yellow mosaic virus* (BYMV)

Bean yellow mosaic virus (BYMV) (genus *Potyvirus*, family *Potyviridae*) particles are long flexuous rods that contain a single-stranded RNA approximately10kb in size (ICTVdb, 2006b). BYMV can infect a wide range of economically important legume species throughout the world and is transmitted by numerous aphid species in a non-persistent manner (ICTVdb, 2006b; Morales, 2005a). The incidence and severity of BYMV symptom expression and economic damage to common bean varies with cultivar, virus strain, plant age, and the prevailing environment, but it has been implicated in minor crop damage as well as devastating epidemics (Morales, 2005a). Important symptoms of BYMV infection of common bean include mosaic, rugosity, leaf deformation, slight pod malformation, and moderate to severe stunting of the entire plant. Due to the non-persistent mode of BYMV transmission, the application of pesticides to control the aphid vectors is ineffective. Deploying cultivars with resistance to BYMV is the most effective, efficient, and ecologically sensitive means to control crop damage.

Resistance to BYMV in *P. vulgaris* has been identified and reported in the literature, but the resistance has not been fully characterized, genetically mapped, widely deployed, nor revisited in decades. Previous research indicated that resistance to BYMV was strain specific and that resistance may be conditioned by different alleles depending on the donor (Bagget and Frazier, 1957; Dickson and Natti, 1968). Three major complimentary recessive genes with modifiers conditioned resistance to the BYMV-Y strain when Great Northern 31 was the donor source of resistance

(Baggett and Frazier, 1957). The same number of genetic factors was implicated in resistance to BYMV derived from the scarlet runner bean (*P. coccineus* L.) accession 2014 (Baggett, 1956.) In contrast, resistance derived from an unnamed scarlet runner bean accession to BYMV has been reported as being conditioned by the single dominant allele, *By*-2 (Dickson and Natti, 1968). Given that the resistance conditioned by *By-2* was inherited as a single dominant allele, and because it had been introgressed into dry bean market classes more recently, research was undertaken to better characterize the sources and inheritance of the resistance conditioned by *By-2*.

1.3 MARKER-ASSISTED SELECTION FOR VIRUS RESISTANCE

Genetic linkage mapping and marker-assisted selection (MAS) have become extremely common in crop plants, and they are arguably essential to modern crop improvement efforts. Over the last two decades, a number of genetic linkage maps were developed in common bean, and a core linkage map was constructed to develop consensus on the location of molecular marker loci (Gepts, 1999; Freyre et al. 2004). Since the first map was developed, linkage mapping has been employed extensively in bean genetic research and MAS has been applied successfully to bean improvement programs (Beaver and Osorno, 2009; Kelly and Miklas, 1999; Miklas et al. 2006). More recently, additional investment and research has greatly improved the number of mapped molecular markers available, the saturation of the core genetic map, and the resolution at which genes can be resolved to their position on linkage groups (Blair et al., 2003; Blair et al., 2009a; Blair et al. 2009b; Hanai et al. 2010). Utilizing the

emerging tools to determine the genetic linkage map location of resistance genes to BYMV and ClYVV will allow for a more thorough and precise characterization of the genetics of resistance to these important virus pathogens. Simultaneously, the development of molecular markers that are linked to the resistance genes may prove to be useful for marker-assisted selection (MAS) and marker-assisted gene pyramiding.

1.4 SUMMARY

The development of a translational genetics approach for resistance to a virus-diseases of common bean will lead to direct impact in that it will facilitate the development of multiple virus-resistant snap beans that are urgently needed in the United States and in many other areas of the world where one, two or all three viruses (ClYVV, BYMV, and CMV) incite crop loss. These viruses have hit snap bean production in the United States particularly hard throughout the past decade, and resistance is desperately needed. The genetic mapping of ClYVV and BYMV resistance loci will greatly facilitate future opportunities for fine-mapping and map-based cloning of potentially novel virus resistance genes. Such research could contribute considerably to the understanding and strategies for deploying the underlying mechanisms of virus resistance in common bean as well as other legumes.

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CHAPTER 2

A SERIES OF EIF4E ALLELES AT THE *Bc-3* LOCUS ARE ASSOCIATED WITH RECESSIVE RESISTANCE TO *Clover yellow vein virus* IN COMMON BEAN[1]

2.1 INTRODUCTION

Clover yellow vein virus (ClYVV) (family *Potyviridae*, genus *Potyvirus*) infection is capable of causing significant damage to leguminous crop and forage plants around the world (CABI/EPPO, 2000). The impact of the disease can be particularly severe in certain interactions with the common bean (*Phaseolus vulgaris* L.) because symptom expression can include severe stunting, prominent mosaic, premature defoliation, systemic necrosis, and in some cases plant death (Provvidenti and Morales, 2005). The impact can be even more acute in the snap bean market class where ClYVV has the potential to devastate marketable yield by causing twisting, distortion, and necrosis of the fresh green pods (Larsen and Myers, 2006; Larsen et al., 2008; Provvidenti and Morales, 2005). The threat of direct economic damage is compounded when pod-distorting strains of the virus appear in processing snap bean production regions as the presence of distorted or necrotic pods above a threshold may result in the rejection of the entire harvest by the processor.

ClYVV epidemics appear to occur sporadically in numerous regions where common beans are grown (CABI/EPPO, 2000; Crnov and Gilbertson, 2001; Dizadji

[1] Hart, J.P., and P.D. Griffiths. 2013. A series of eIF4E alleles at the *Bc-3* locus are associated with recessive resistance to *Clover yellow vein virus* in common bean. Theoretical and Applied Genetics. 126:2849-2863.

and Shahraeen, 2011; Larsen and Myers, 2006; Ortiz et al., 2009; Provvidenti and Shroeder, 1973; Sasaya et al., 1997; Tu, 1980; Tu, 1988), but the increased frequency of an aphid-transmitted virus disease complex of snap beans in the Great Lakes Region of the United States over the past decade has been cause for concern (Larsen et al., 2002; Larsen et al., 2008; Shah et al., 2006). Processing snap bean is a major vegetable crop in this region where the farm-gate value alone exceeds $100 million (USDA-NASS, 2011). A number of aphid species are present in the region that are potential vectors of ClYVV (Nault et al., 2004), but the increased incidence of ClYVV (and other viruses) in snap bean production has been associated with the accidental introduction of the soybean aphid (*Aphis glycines* Matsumura) to the United States in 2000 or earlier (Hill et al., 2001; Ragsdale et al., 2004). Aphid vectors transmit ClYVV in a nonpersistent manner where the virus may be acquired and transmitted within seconds of stylet penetration (Nault, 1997; Nault et al., 2004). For this reason and others, nonpersistent aphid-transmitted viruses can be exceedingly difficult to control. Host plant resistance, if available, is the most effective, efficient, and ecologically sensitive means to reduce the potential for crop damage.

Genetic variation for resistance to ClYVV in common bean has been identified. The *by-3* gene was first identified in GN 1140 where it provided homozygous recessive resistance to a number of isolates of the severe (pod-distorting) strain of *Bean yellow mosaic virus* (BYMV-S) (Provvidenti and Shroeder, 1973). The *by-3* gene symbol was subsequently revised to *cyv* by Provvidenti (1987) to reflect taxonomic revision of BYMV-S to ClYVV (as proposed by Bos et al., 1977, substantiated by Uyeda et al., 1991 and Tracy et al., 1992). A single gene in Kentwood

that was donated by Clipper conditioned homozygous recessive resistance to a necrotic strain of ClYVV previously typed as BYMV-N from Ontario, Canada (Tu, 1980; Tu, 1983). Genetic analysis of the resistance present in GN UI 31 to an isolate of BYMV-S from Oregon revealed that two recessive genes were required for resistance (Tatchell et al., 1985). Subsequent research demonstrated that the recessive *cyv* gene present in GN 1140 was allelic with the ClYVV resistance present in the cultivars Kentwood, Harokent, Imuna, and Amanda (Park and Tu, 1991).

The mechanism of resistance present in Jolanda to the ClYVV no. 30 strain from Japan was characterized by employing a ClYVV vector that expressed green fluorescent protein (pClYVV/C3-S65T) (Sato et al., 2003). The results demonstrated that the resistance phenotype operated on the single cell level by completely inhibiting ClYVV replication in healthy cells (Sato et al., 2003). A spontaneous resistance-breaking mutant of the virus (ClYVV-Br) was then utilized to develop chimeric clones and to map the avirulence determinant of the virus. The results suggested that the viral genome-linked protein (VPg) was the avirulence determinant (Sato et al., 2003) similar to that observed in other plant-potyvirus pathosystems (Hjulsager et al., 2002; Keller et al., 1998; Nicolas, 1997). Genetic analysis revealed that the resistance was conditioned by a single recessive gene which was separate from the potyvirus resistance conferred by the *Bean common mosaic virus* (BCMV) resistance genes *I*, *bc-1*, and *bc-u* which were also present in Jolanda (Drijfhout, 1978; Sato et al., 2003). The resistance gene was designated *desc* (determinant of susceptibility to ClYVV) to reflect the hypothesis that it was based on the absence of a factor necessary for viral infection and replication, and possibly analogous to the phenomenon of recessive

resistance conditioned by mutations in eukaryotic translation initiation factor 4E (eIF4E) and its isoform (eIF(iso)4E) as elucidated in *Capsicum* spp. (Ruffel et al., 2002) and *Arabidopsis thaliana* (Lellis et al., 2002) respectively. Allelism testing with additional ClYVV no. 30 resistant bean cultivars Evolutie and Imuna revealed that they possessed the same *desc* resistance (Sato et al., 2003). The allelic relationship between *desc* and the previously identified *cyv* gene reported in Imuna (Park and Tu, 1991) was not established. In addition, it appears the hypothesis that mutations in eIF4E or eIF(iso)4E present in Jolanda conferred resistance to ClYVV no. 30 was never tested.

A novel strain of ClYVV from Wisconsin (ClYVV-WI) associated with an emerging U.S. Great Lakes virus disease complex was recently discovered and characterized (Larsen et al., 2008). ClYVV-WI infection caused severe symptoms in a collection of 63 commercial snap bean cultivars and other common bean genotypes, including the previously reported resistant cultivars Imuna and Jolanda (Larsen et al., 2008). Complete resistance to ClYVV-WI, as well as the New York (ClYVV-NY) and Oregon (ClYVV-OR) strains, was identified in all common bean genotypes that possessed the recessive *bc-3* gene (USLK-1, USLK-2, USDK-4, USDK-5, USWK-6, USCR-7, USCR-8, USCR-9, and Raven) with the exception of IVT 7214 (Larsen et al. 2008). The potential involvement of *bc-3* is particularly interesting given that its combination with the *bc-u*, and/or *I* gene(s) (Ali, 1950; Collmer et al., 2000) confers resistance to all known strains of the related potyviruses *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) worldwide (Drijfhout, 1978; Kelly et al. 1995; McKern et al., 1992), except the recombinant NL 3 K strain of

BCMV/BCMNV (Larsen et al., 2005b).

The *bc-3* gene is one of six recessive alleles (*bc-u*, *bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc*-3) at four independent loci, that along with the dominant allele of the *I* gene act in allele-specific combinations to condition resistance, and to define seven pathogenicity groups of BCMV and BCMNV (for reviews, see Drijfhout, 1978; Drijfhout et al., 1978; Kelly et al., 1995; Morales, 2005; Mukeshimana et al., 2005). Full expression of the resistance conditioned by the five strain specific alleles (*bc-1*, *bc-1*^{2}, *bc-2*, *bc-2*^{2}, and *bc-3*) requires the homozygous recessive state of the strain nonspecific *bc-u* gene when the dominant allele of the *I* gene is absent (Drijfhout, 1978). When the *I* gene is present in *Bc-u* genotypes, all resistance alleles are fully expressed except for those at the *bc-2* locus (Kelly et al., 1995). The *i*, *Bc-u*, *bc-3* combination is known to condition resistance to all known strains of BCMNV, but not to all strains of BCMV (US1, US3, US7), although another uncharacterized gene that interacts with *bc-3* may be responsible for this resistance spectrum (Miklas et al., 1998). The allele combinations *I*, *bc-3* as well as *bc-u*, *bc-3,* condition resistance to all known strains of BCMV, BCMNV (except NL 3 K), and all known strains of ClYVV (Larsen et al., 2008). Due to the critical importance of the resistance spectrum conditioned by *bc-3,* and its recessive inheritance, common bean breeders and geneticists have invested in the development and employment of molecular markers to map *bc-3* to common bean chromosome 6, and to enable marker-assisted selection and introgression of *bc-3* (Johnson et al., 1997; Kelly et al., 2003; Mukeshimana et al., 2005; Naderpour et al., 2010; Pedrosa-Harrand et al., 2008).

Revolutionary advances in the understanding of the molecular genetic and

functional basis for resistance to the *Potyviridae* have lead to the conclusion that naturally occurring amino acid substitutions in specific regions of eIF4E and eIF(iso)4E result in the numerous and effective recessive resistance genes common across many important monocot and dicot crop plant-potyvirus pathosystems (see reviews, Diaz-Pendon et al., 2004; Kang et al., 2005a; LeGall et al., 2011; Robaglia and Caranta, 2006; Truniger and Aranda, 2009; Wang and Krishnaswamy, 2012). Recognizing this, eIF4E, eIF(iso)4E, and the novel cap binding protein (nCBP) were cloned and sequenced from a collection of nine common bean genotypes that represented eight different allele combinations of the *I* and *bc-* resistance genes (Naderpour et al., 2010). Predicted amino acid substitutions were discovered in a variant of *P. vulgaris* eIF4E (PveIF4E^2), which was cloned exclusively from four genotypes that possessed the *bc-3* resistance allele. Alignment of PveIF4E^2 with eIF4E variants that have been confirmed to condition resistance to potyviruses in *Capsicum annuum* (Kang et al., 2005b; Ruffel et al., 2002), *Lactuca sativa* (Nicaise et al., 2003) and *Pisum sativum* (Gao et al., 2004) revealed analogous features of amino acid substitutions in the predicted cap binding pocket of the protein (Naderpour et al., 2010). A cleaved amplified polymorphic sequence (CAPS) marker diagnostic for *PveIF4E^2* cosegregated with *bc-3* conditioned resistance to BCMV strain NL 1 (NL 1) in a segregating F_2 population of 96 individuals (Naderpour et al., 2010). Though not confirmed by direct complementation testing, these results provided the initial evidence towards mutated eIF4E alleles as the molecular basis for *bc-3*.

In light of the possibility that *bc-3* resistance to ClYVV may also be confirmed by a mutant eIF4E allele, and the immediate practical need to deploy resistance to

ClYVV in the Great Lakes Region of the U.S., a more thorough understanding of the available genetic variation for resistance was needed. The objectives of this research were to evaluate, identify, and characterize the ClYVV resistance present in 21 informative common bean genotypes and establish the allelic relationships of all three previously reported recessive resistance genes to ClYVV (*cyv*, *desc*, and *bc-3*). Subsequent objectives were to further examine the potential association of predicted amino acid substitutions in PveIF4E with resistance to ClYVV, and then to develop a set of single nucleotide polymorphism (SNP)-based markers for rapid allelic discrimination and marker-assisted selection of the recessive potyvirus resistance alleles at the *Bc-3* locus.

2.2 MATERIALS AND METHODS

2.2.1 Plant materials and population development

A panel of 21 common bean genotypes was assembled to include entries reported to possess the putatively independent recessive resistance genes to ClYVV (*cyv*, *desc*, or *bc-3*) as well as additional entries with known and/or demonstrated virus interaction phenotypes (Table 2.1). Five of the entries were snap bean genotypes (Baby Bop, Laureat, Paloma, Polder, Sonesta) that were identified to be resistant to ClYVV-NY as the result of a screen of cultivars (Hart and Griffiths, unpublished). Populations were developed for cosegregation analysis and allelism testing. Midnight, the recurrent susceptible parent used in the development of the ClYVV resistant cultivar Black Knight was crossed to Black Knight to create F_1 and F_2 populations for cosegregation analysis. Black Knight is nearly isogenic with Midnight but possesses

Table 2.1. Common bean genotypes, sources, market classes, previously reported resistance genes to *Clover yellow vein virus* (ClYVV), and their respective responses to the NY strain of ClYVV (ClYVV-NY) and the NL 3 D strain of *Bean common mosaic necrosis virus* (NL 3 D).

Genotype	Accession†	Market Class	ClYVV Resistance‡	Response to ClYVV-NY†††	Response to NL 3 D†††
Dubbele Witte	PI 377736	Fresh Snap	-	sS, VN, sM, TN	S, Ld, M
Midnight	PI 550032	Black	-	sS, VN, sM	VN, SN, D
Hystyle	PI 550288	Processing Snap	-	sS, VN, sM, TN	VN, SN, D
GN 1140	PI 549667	Great Northern	*cyv*§	NS	mM
Black Knight	CU	Black	*cyv* ¶	NS	VN, SN, D
Jolanda	G 7591	Fresh Snap	*desc#*	NS	NLL, VN, Ld, M
Amanda	PI 599026	Fresh Snap	*cyv*††	NS	lVN
Baby Bop	PI 642144	Processing Snap	-	NS	VN, SN, D
Laureat	PI 550261	Processing Snap	-	NS	VN, SN, D
Paloma	CU	Fresh Snap	-	NS	VN, SN, D
Polder	CU	Fresh Snap	-	NS	VN, SN, D
Sonesta	CU	Fresh Snap	-	NS	VN, SN, D
Imuna	PI 326420	Fresh Snap	*cyv*††, *desc#*	NS	mM
Evolutie	W6 42706	Fresh Snap	*desc#*	NS	VN, SN, D
Clipper	PI 278776	Navy	*cyv*‡‡	NS	S, Ld, M
CY-10 S_4	CU	Processing Snap	*cyv*	NS	VN, SN, D
IVT 7214	PI 602987	Expt. Line	*bc-3*§§	NS	NS
Raven	MSU	Black	*bc-3*¶¶	NS	NS
B/R RIL105-25	MSU	Navy	*bc-3*	NS	NS
USWK-6	PI 618815	White Kidney	*bc-3*##	NS	NS
USWKH x H S_4	CU	Expt. Line	*bc-3*	NS	NS

† Accessions that begin with 'PI' and 'W6' were sourced from the U.S. National Plant Germplasm System, 'G' International Center for Tropical Agriculture, 'CU' Cornell University, and 'MSU' Michigan State University.

‡Resistance genes cited in the following references: § Provvidenti and Shroeder 1973, ¶ Halseth et al. 1998, # Sato et al. 2003, †† Park and Tu 1991, ‡‡ Tu 1983, §§ Drijfhout 1978, ¶¶ Kelly et al. 1994, ## Miklas et al. 2002.

††† Symptom legend: D = plant death, Ld = leaf distortion, M = mosaic, mM = mild mosaic, sM = severe mosaic, NLL = necrotic local lesions, NS = no symptoms, S = stunting, sS = severe stunting, SN = systemic necrosis, VN = vein necrosis, lVN = localized vein necrosis.

the *cyv* resistance allele (Halseth et al., 1998) from GN 1140 (Provvidenti, 1987; Provvidenti and Shroeder, 1973). Black Knight was also crossed to Raven (Kelly et al., 1994) that possesses the *I*, *bc-3* allele combination to develop F_1 and F_2 populations for cosegregation analysis and allelism testing. B/R RIL 105-25, a breeding line developed at Michigan State University (East Lansing, MI) that possesses *I*, *bc-3* from Raven was crossed to the ClYVV resistant cultivar Clipper that possesses *cyv* (Tu, 1983) to develop F_1, F_2, and $F_{2:3}$ populations for allelism testing. The breeding line CY-10 S_4 was crossed to USWK x H S_4 to develop F_1 and F_2 populations for allelism testing. CY-10 S_4 is a BC_8S_4 line developed at Cornell University (CU) New York State Agricultural Experiment Station (NYSAES) (Geneva, NY) through the introgression of *cyv* from Clipper into the susceptible recurrent parent Hystyle. USWKH x H S_4 is a BC_2S_4 line developed at CU through introgression of *bc-3* from USWK-6 into the susceptible recurrent parent Hystyle. USWK-6 is a breeding line developed by the USDA-ARS that possesses *I*, *bc-3* (Miklas et al., 2002).

Additional F_1 populations were developed to investigate allelic relationships through complementation testing. Amanda (*cyv*) was crossed to Imuna (*cyv*/*desc*) and Jolanda (*desc*) was crossed to Clipper (*cyv*) to develop F_1 populations. All F_1 hybrid plants were confirmed heterozygous by morphology, by allele-specific molecular marker assays, or both. The molecular marker assays are described in the 'sequence analysis and design of allele-specific assays' section of the methods below.

Seeds of all experimental material were sown in 'Cornell mix' (Boodley and Sheldrake, 1972) in greenhouses at CU NYSAES, where routine watering,

fertilization, and insect control measures were employed. Plant growth conditions were 24° C day /21°C night with a 14-hr photoperiod. Supplemental lighting was produced by 1000 W metal halide bulbs (SunSystem III, Sunlight Supply, Inc., USA) to provide a rate of 300 μM m^2 s^1 of photosynthetically active radiation at bench level. All of the plants used in the experiments were grown in 14.6 cm^2 pots except for the F_2 derived F_3 families ($F_{2:3}$) which were grown in 18 cell flats (Speedling Inc., USA) with cells of 10 cm^2.

2.2.2 Virus isolates, inoculation, and resistance evaluation

ClYVV-NY was obtained from the Rosario Provvidenti collection at CU NYSAES and confirmed to be a pure isolate by host range and symptomatology, and RT-PCR (Provvidenti and Shroeder 1973; Shail et al., 2007). The reaction of the common bean BCMV/ BCMNV host differential groups and genotypes to ClYVV-NY is presented in Appendix 2.1. ClYVV-NY is identical to the strain employed by Larsen et al. (2008). ClYVV-NY was maintained in the susceptible snap bean cultivar Hystyle. The NL 3 D strain (Drijfhout 1978, Larsen et al., 2005b) was obtained from Dr. Phillip Miklas of the USDA-ARS (Prosser, WA) and was maintained in the cultivar Dubbele Witte. The virus strains were maintained by periodical transfer (~ 3 weeks) to the expanding primary leaves of seedlings of the susceptible cultivars by mechanical inoculation. Virus inoculum was prepared by homogenizing newly expanded, symptomatic, virus-infected trifoliate leaves (1:10 w/v) in cold 10mM phosphate buffer (3mM K_2PO_4, 7mM Na_2HPO_4, pH 7.0) with a mortar and pestle. The homogenate was applied with the pestle by gently rubbing newly expanded primary

leaves of seedlings (7-10 days after planting) that had been dusted with carborundum (silicon carbide, 400 mesh, Sigma-Aldrich). The inoculated plants were then lightly rinsed with water. All plants were inoculated again two days after the first inoculation, and plants that did not display virus symptoms 10 days post the first inoculation (dpi) were re-inoculated to prevent escape.

At least twenty inoculated plants of each of the 21 genotypes included in the panel were inoculated and evaluated in three separate experiments. The plants were examined daily for virus symptoms, and symptom expression was evaluated in comparison to non-inoculated, susceptible, and resistant controls, and recorded 10, 21, 30, and 45 dpi. Symptom expression in response to inoculation with ClYVV-NY and NL 3 D was evaluated qualitatively following the descriptors defined in the caption of Table 2.1. At 21 dpi, leaf tissue from newly expanded trifoliate leaves was collected for virus detection by enzyme-linked immunosorbent assay (ELISA). The ELISA was performed according to the manufacturer's instructions [(for ClYVV-NY:ClYVV-C81 & Pratt, AC Diagnostics) (for NL 3 D: Potyvirus Group, Agdia)]. The absorbance at OD_{405nm} was measured with a multi-mode microplate reader (Synergy 2, Biotek Instruments) following the final incubation and after two additional one-hour intervals. Absorbance reads that were at least two times greater than that of the healthy negative control were considered to be positive for presence of the virus.

2.2.3 Cloning and sequencing of *P. vulgaris eIF4E*

Coding sequences of *PveIF4E* for each genotype in Table 2.1 were obtained as follows. Total RNA was isolated with an Ambion RNAqueous-4PCR Kit (Life

Technologies) following the manufacturer's instructions. The RNA was eluted into 30 μL of elution buffer and quantified by spectrophotometry so that 1 μg of RNA could be used for cDNA synthesis. Reverse transcription was performed with an Ambion RETROscript First Strand Synthesis Kit following the manufacturer's instructions (Life Technologies). Oligonucleotide primers capable of amplifying the entire *PveIF4E* coding sequence were designed prior to the release of the *P. vulgaris* genome sequence, and thus were designed to anneal to the extremities of the *eIF4E* coding sequences of pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.). The primer pair was designed using Primer 3 (Rozen and Skaletsky, 2000); the forward primer was designated Leg4E-F (5'-ATG GTT GTA GAA GAT ACC C-3') and the reverse primer, Leg4E-R (5'- TCA TAC AAC GTA TTT ATT TTT AGC-3'). PCR amplification was performed in 20 μL reactions containing 0.5 μL of cDNA, 5 pmol of each primer, and 1.5 units of Platinum Taq DNA Polymerase High Fidelity (Life Technologies) using an Eppendorf Gradient Master Cycler (Eppendorf). The PCR program consisted of 1 denaturation cycle of 3 min at 95° C followed by 40 cycles of 30 s each of 94° C, 53° C, and 72° C. Aliquots of the PCR products were resolved by 1.5% TAE agarose gel electrophoresis and visualized with ethidium bromide under UV-light to confirm that amplicons had the expected size of 693 bp. PCR products were then purified using a QIAquick PCR purification kit (Qiagen).

Two independently amplified PCR products from each genotype were cloned into the pCR4 vector using the TOPO TA Cloning Kit for Sequencing (Life Technologies). DNA was isolated from liquid cultures of two single colony clones in selective media using the QIAprep Spin Miniprep kit (Qiagen). Nucleotide sequencing

was performed with an Applied Biosystems Automated 3730 DNA Analyzer using Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase (Life Technologies) at the CU Life Sciences Core Laboratories Center, Ithaca, NY.

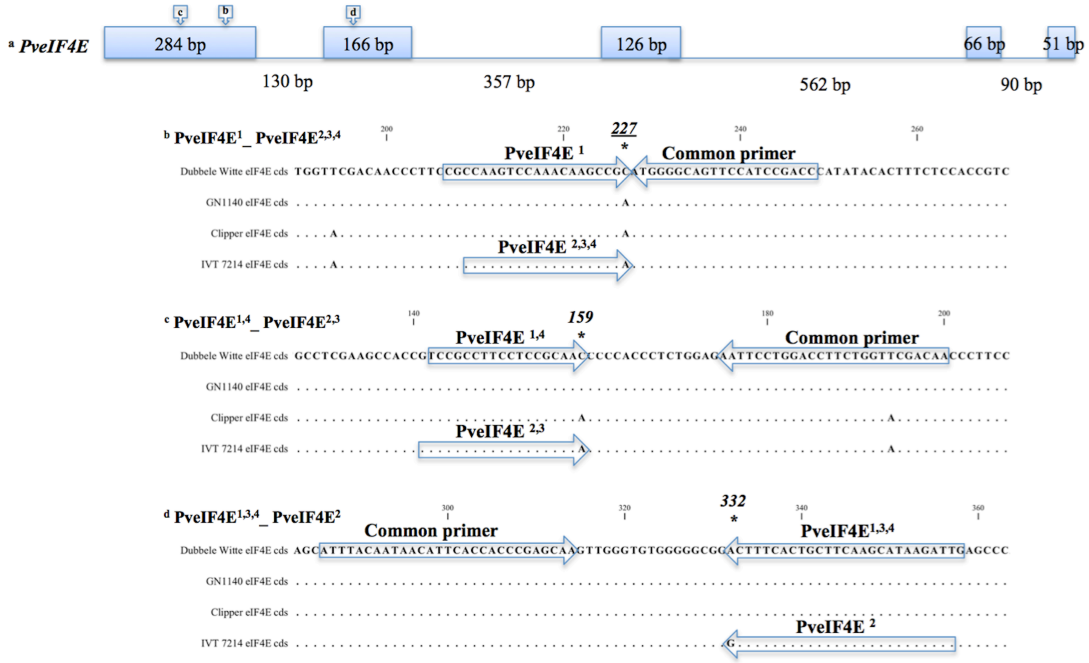
2.2.4 Sequence analysis and design of allele-specific assays

The *PveIF4E* coding sequences and deduced proteins were aligned to detect non-synonymous SNPs and amino acid substitutions. Multiple sequence alignment by ClustalW was performed with the CLC Main Workbench (CLC Bio) software. *PveIF4E* coding sequences were also aligned to the *Glycine max* (L.) Merr. genomic sequence (Schmutz et al., 2010) with the 'est2genome' model of the Exonerate multiple sequence alignment program (Slater and Birney, 2005) to predict the intron-exon boundaries of *PveIF4E*. Non-synonymous SNPs that corresponded with defined virus resistance phenotypes were chosen for the design of KBiosciences Competitive Allele-Specific PCR (KASPar) SNP assays (LGC-KBiosciences) (Fig. 2.1). At least 50 bp of sequence flanking both sides of the targeted SNPs was submitted to the KBiosciences KASPar By Design service and the primers in 2.2 were synthesized for the assays.

Table 2.2. Assay ID, target SNPs, and primer sequences of the KASPar assays employed.

KASPar assay ID	SNP	Primer	Sequence
PveIF4E[1] __PveIF4E[2,3,4]	**C**227**A**	PveIF4E[1] Allele (**C**_VIC)	CGCCAAGTCCAAACAAGCCGC
		PveIF4E[2,3,4] Allele (**A**_FAM)	CGCCAAGTCCAAACAAGCCGA
		Common	GGTCGGATGGAACTGCCCCAT
PveIF4E[1,4] __PveIF4E[2,3]	**C**159**A**	PveIF4E[1,4] Allele (**C**_VIC)	GTCCGCCTTCCTCCGCAAC
		PveIF4E[2,3] Allele (**A**_FAM)	CGTCCGCCTTCCTCCGCAAA
		Common	TGTCGAACCAGAAGGTCCAGGAATT
PveIF4E[1,3,4] __PveIF4E[2]	**A**332**G**	PveIF4E[1,3,4] Allele (**A**_ VIC)	CAATCTTATGCTTGAAGCAGTGAAAGT
		PveIF4E[2] Allele (**G**_FAM)	AATCTTATGCTTGAAGCAGTGAAAGC
		Common	ATTTACAATAACATTCACCACCCGAGCAA

Fig. 2.1. Schematic of genomic *PveIF4E* where boxes correspond to exons, lines correspond to introns, and the relative positions of the non-synonymous SNPs targeted for the development of KASPar assays are indicated by boxes with arrows. The KASPar assays described in 2.2 are illustrated below.



2.2.5 DNA extraction and KASPar Assays

Genomic DNA for use in KASPar assays was isolated from ~50 mg of tissue from young trifoliate leaves according to Afanador et al. (1993). DNA was checked for purity by spectrophotometry [NanoDrop ND-1000, (Thermo Scientific)] and was quantified using Quant-iT PicoGreen (Life Technologies) and a multi-mode microplate reader [Synergy 2 (Biotek Instruments)]. DNA was diluted with nuclease free water to 5ng/μL. KASPar assays were performed according to the manufacturer's instructions (Robinson and Holmes, 2011) in 8μL reaction volumes containing 4 μL of DNA, 4 μL of 2x KASP reaction mix, and 0.11 μL of the assay mix containing the common primer and the fluor-labeled allele- specific primers. PCR amplification consisted of one 15 min cycle at 94° C, followed by 10 cycles of 94° C for 20 s, 65 - 57° C for 60 s (dropping 0.8° C per cycle), and then 30 cycles of 94° C for 20 s, and 57° C for 60 s using an Eppendorf Gradient Master Cycler PCR machine (Eppendorf). Fluorescent endpoint analysis was performed with a ViiA7 Real-Time PCR system (Life Technologies). Fluorescent endpoint analysis data was analyzed with the 'genotyping experiment' module of the ViiA7 Software package (Life Technologies) and allele calls were assigned to the samples automatically by the software. In some cases, interaction with the software to infer an allele call, or to remove a failed reaction, was necessary.

2.3 RESULTS

2.3.1 Allelism tests demonstrated that *cyv*, *desc*, and *bc-3* are allelic

The lack of symptoms (Table 2.1) and negative ELISA results (data not shown) confirmed that the genotypes included in the panel that possessed *cyv*, *desc*, and *bc-3* all conditioned resistance to ClYVV-NY. These results validated the use of ClYVV-NY to evaluate potential allelic relationships. All F_1, F_2, and $F_{2:3}$ populations derived from the crosses between ClYVV resistant parents possessing either *cyv*, *desc*, or *bc-3* were uniformly resistant based on the absence of symptoms (Table 2.3) and negative ELISA results. In total, 90 individuals from five separate F_1 populations, 289 individuals from two separate F_2 populations, and 132 $F_{2:3}$ lines (1188 individuals) were resistant to ClYVV-NY. The failure of complementation to restore susceptibility provides clear evidence that *cyv*, *desc*, and *bc-3* are allelic and therefore map to the same genetic locus on chromosome 6.

2.3.2 Resistance to ClYVV-NY in *P. vulgaris* is associated with an allelic series at *PveIF4E*

The complete coding sequences of *PveIF4E* were obtained from each of the 21 genotypes in Table 2.1 and were examined for non-synonymous single nucleotide polymorphisms (Table 2.4; for full-length alignment see Appendix 2.2 and 2.3). The Leg4E primer pair produced a single amplicon of the expected size of 693 bp for all cDNA templates. BLASTn and BLASTp (http://blast.ncbi.nlm.nih.gov/) queries of the sequenced amplicons and the predicted amino acid sequences from Dubbele Witte and Raven confirmed identity with *PveIF4E*[1] (EF571267) and *PveIF4E*[2] (EF571273)

respectively, previously cloned and sequenced from the same genotypes (Naderpour et al., 2010). This gene corresponds to the locus Phvul.006G168400 on chromosome six of the V1.0 release of the *P. vulgaris* genome [Phaseolus vulgaris V1.0 (DOE-JGI and USDA-NIFA, 2013)].

The complete *PveIF4E* coding sequences from Midnight and Hystyle, two genotypes (in addition to Dubbele Witte) that were susceptible to ClYVV-NY, and displayed the unprotected *I* gene response of systemic necrosis to NL 3 D (Kelly et al., 1995), were identical to *PveIF4E*^{1}. The complete *PveIF4E* coding sequences from IVT 7214, B/R RIL 105-25, USWK-6, and USWKH x H S_4, four genotypes (in addition to Raven) that were resistant to ClYVV-NY and NL 3 D as conditioned by *bc-3* were identical to *PveIF4E*^{2}. *PveIF4E*^{2} differed from *PveIF4E*^{1} by four SNPs that each resulted in predicted amino acid substitutions at positions 53 (N/K), 65 (F/Y), 76 (A/E), and 111 (D/G) of the predicted protein (Table 2.4). *PveIF4E*^{2} was associated with homozygous recessive resistance to NL 1 as conditioned by the *bc-3* gene in previous cosegregation analysis (Naderpour et al., 2010).

Two novel *PveIF4E* mutant alleles were identified, *PveIF4E*^{3}, and *PveIF4E*^{4}, that were identical for some, but not all of the SNPs and predicted amino acid substitutions of *PveIF4E*^{2} (Table 2.4). These novel mutant alleles were exclusive to genotypes with resistance to ClYVV-NY and susceptibility to NL 3 D. *PveIF4E*^{3}, first cloned and sequenced from Clipper, also present in CY-10 S_4, Evolutie, and Imuna, possessed three of the four predicted amino acid substitutions present in *PveIF4E*^{2} at positions 53 (N/K), 65 (F/Y), and 76 (A/E) respectively. *PveIF4E*^{4}, first cloned and sequenced from GN 1140, also present in Black Knight, Jolanda, Amanda, Baby Bop,

Table 2.3. Resistance to ClYVV-NY in common bean parental genotypes and populations tested for allelism

Genotype	ClYVV Resistance	Population	No. Plants / Lines	Phenotype (ClYVV-NY)
Clipper	*cyv*	Parent 1	18	Resistant
B/R RIL 105-25	*bc-3*	Parent 2	18	Resistant
Clipper x B/R RIL 105-25	*cyv bc-3*	F_1	18	Resistant
Clipper x B/R RIL 105-25		F_2	132	Resistant
Clipper x B/R RIL 105-25		$F_{2:3}$	132†	Resistant
CY-10 S_4	*cyv*	Parent 1	18	Resistant
USWKH x H S_4	*bc-3*	Parent 2	18	Resistant
CY-10 S_4 x USWKH x H S_4	*cyv bc-3*	F_1	18	Resistant
CY-10 S_4 x USWKH x H S_4		F_2	157	Resistant
Black Knight	*Cyv*	Parent 1	18	Resistant
Raven	*bc-3*	Parent 2	18	Resistant
Black Knight x Raven	*cyv bc-3*	F_1	18	Resistant
Black Knight x Raven		F_2	40	Resistant
Amanda	*cyv*	Parent 1	18	Resistant
Imuna	*cyv (desc)*	Parent 2	18	Resistant
Amanda x Imuna	*cyv cyv(desc)*	F_1	18	Resistant
Jolanda	*desc*	Parent 1	18	Resistant
Clipper	*cyv*	Parent 2	18	Resistant
Jolanda x Clipper	*desc cyv*	F_1	18	Resistant

† 9 plants per $F_{2:3}$ line tested.

Laureat, Paloma, Polder, and Sonesta, possessed only one of the four predicted amino acid substitutions present in *PveIF4E*^{2} at position 76 (A/E). This result strongly suggests that the A76E mutation is a determinant for resistance to ClYVV-NY.

Only genotypes that possessed *PveIF4E*^{2} and the predicted D111G mutation were resistant to NL 3 D (Table 2.4). Similarly, this result strongly suggests that the D111G mutation is a determinant for resistance to NL 3 D. The pattern of exclusive non-synonymous nucleotide substitutions present in *PveIF4E*^{2}, *PveIF4E*^{3}, and *PveIF4E*^{4} are analogous to the patterns of polymorphism in the critical area of eIF4E

for cap-binding and recognition where amino acid substitutions have been associated with, or directly demonstrated to condition potyvirus resistance in an extensive range of crop plants (Diaz-Pendon et al., 2004; Kang et al., 2005a; LeGall et al., 2011; Robaglia and Caranta, 2006; Truniger and Aranda, 2009; Wang and Krishnaswamy, 2012).

2.3.3 Allele-specific genotyping and cosegregation analysis

In order to progress from polymorphism discovery to allele-specific molecular markers as rapidly as possible, three KASPar SNP genotyping assays were developed to differentiate and confirm the allelic state of all of the *PveIF4E* alleles (Table 2.2). The assays were employed to investigate genotype-phenotype relationships, to confirm allelism, and to validate the utility of the assays for allelic discrimination across the germplasm.

KASPar assay 'PveIF4E^{1}__PveIF4E^{2,3,4}' targets the SNP227 (C227A) mutation that corresponds to the predicted A76E amino acid substitution that is present in the *PveIF4E*^{2,3,4} alleles, all associated with resistance to ClYVV-NY (2.4). This assay was employed to investigate genotype-phenotype relationships in parental, F_1, and F_2 populations of the Midnight x Black Knight cross (Table 2.5). An example of the allele calls made by the software is illustrated in Appendix 2.5 for 88 Midnight x Black Knight F_2 individuals. The genotypic and phenotypic segregation ratios fit that expected of a single recessive locus, and the homozygous state of the mutant allele (AA) was in complete linkage with the resistance phenotype in 49 F_2 individuals (Table 2.5).

Table 2.4. Resistance of 21 common bean genotypes to ClYVV-NY and NL 3 D and their corresponding nucleotide polymorphisms and predicted amino acid substitutions in *PveIF4E*

			Position of nucleotide (nt) polymorphism and amino acid (**aa**) substitutions ‡								
Genotype	ClYVV-NY†	NL 3 D†	nt 159	**aa 53**	nt 194	**aa 65**	nt 227	**aa 76**	nt 332	**aa 111**	*PveIF4E* alleles§
Dubbele Witte	S	S	C	**N**	T	**F**	C	**A**	A	**D**	*PveIF4E*^{1} (¶)
Midnight	S	S	-	-	-	-	-	-	-	-	
Hystyle	S	S	-	-	-	-	-	-	-	-	
GN 1140	*r* (*cyv*)	S	-	-	-	-	A	**E**	-	-	
Black Knight	*r* (*cyv*)	SN	-	-	-	-	A	**E**	-	-	
Jolanda	*r* (*desc*)	VN	-	-	-	-	A	**E**	-	-	*PveIF4E*^{4}
Amanda	*r* (*cyv*)	VN	-	-	-	-	A	**E**	-	-	
Baby Bop	*r*	SN	-	-	-	-	A	**E**	-	-	
Laureat	*r*	SN	-	-	-	-	A	**E**	-	-	
Paloma	*r*	SN	-	-	-	-	A	**E**	-	-	
Polder	*r*	SN	-	-	-	-	A	**E**	-	-	
Sonesta	*r*	SN	-	-	-	-	A	**E**	-	-	
Imuna	*r* (*cyv/desc*)	S	A	**K**	A	**Y**	A	**E**	-	-	
Evolutie	*r* (*desc*)	SN	A	**K**	A	**Y**	A	**E**	-	-	*PveIF4E*^{3}
Clipper	*r* (*cyv*)	S	A	**K**	A	**Y**	A	**E**	-	-	
CY-10 S_4	*r* (*cyv*)	SN	A	**K**	A	**Y**	A	**E**	-	-	
IVT 7214	*r* (*bc-3*)	*r* (*bc-3*)	A	**K**	A	**Y**	A	**E**	G	**G**	
Raven	*r* (*bc-3*)	*r* (*bc-3*)	A	**K**	A	**Y**	A	**E**	G	**G**	
B/R RIL105-25	*r* (*bc-3*)	*r* (*bc-3*)	A	**K**	A	**Y**	A	**E**	G	**G**	*PveIF4E*^{2} (#)
USWK-6	*r* (*bc-3*)	*r* (*bc-3*)	A	**K**	A	**Y**	A	**E**	G	**G**	
USWKH x H S_4	*r* (*bc-3*)	*r* (*bc-3*)	A	**K**	A	**Y**	A	**E**	G	**G**	

† 'S' denotes 'susceptible', 'SN' denotes 'systemic necrois', 'VN' denotes 'vein necrosis' 'r' denotes 'resistant'; confirmed by screening 5 to 10 plants of each cultivar in three independent experiments, with 'r' confirmed by ELISA.

‡ A dash (-) in the table represents an identical nt or aa as *PveIF4E*^{1}.

§ *PveIF4E* alleles are numbered by superscript in the order in which they were discovered.

¶ *PveIf4E*^{1} first cloned and sequenced from Dubbele Witte and others in Naderpour et al. 2010.

PveIF4E^{2} first cloned and sequenced from Raven in Naderpour et al. 2010.

KASPar assay 'PveIF4E^{1,3,4}__PveIF4E^{2}' targets the SNP332 (A332G) mutation that corresponds to the predicted D111G amino acid substitution present only in *PveIF4E*^{2} and that is associated with *bc-3* resistance to ClYVV-NY, ClYVV-WI (Larsen et al., 2008), NL 3 D, and NL 1 (Naderpour et al., 2010) (Table 2.4). This assay was employed to investigate genotype-phenotype relationships in the parental, F_1, and F_2 populations of the Black Knight x Raven cross (Table 2.6). The genotypic and phenotypic segregation ratios fit that expected for a single recessive locus, and the homozygous state of the mutant allele (GG) was in complete linkage with the resistance phenotype in 50 F_2 individuals (Table 2.6).

KASPar assay 'PveIF4E^{1,3,4}__PveIF4E^{2}'(A332G) was also employed to demonstrate its ability to discriminate alleles at the molecular genetic level in the parental, F_1, and F_2 allelism testing populations of the Clipper x B/R RIL 105-25 cross where all phenotypes were identical (Table 2.7). The genotypic segregation ratio fit the expectation of a single locus (Table 2.7). The phenotypic segregation ratio could not be ascertained because the population was inoculated and evaluated only for resistance to ClYVV-NY. KASPar assay 'PveIF4E^{1,4}__PveIF4E^{2,3}' (C159A) was designed for the purposes of allelic discrimination and haplotype analysis. The KASPar assays were employed for haplotype analysis across the 21 common bean genotypes. Table 2.8 demonstrates the assays' ability to discriminate between the *PveIF4E* alleles, and to ultimately predict the resistance allele and spectrum. The KASPar assays consistently and accurately identified the correct haplotypes for all of the *PveIF4E* alleles.

Table 2.5. Cosegregation of KASPar SNP PveIF4E[1] (**C**) allele with susceptibility and KASPar SNP PveIF4E[2,3,4] (**A**) allele with resistance to ClYVV-NY as conditioned by *cyvcyv* in the Midnight x Black Knight populations

			PveIF4E[1]__PveIF4E[2,3,4]			
Genotype	Population	ClYVV Resistance	SNP (**C**227**A**)	No.†	Phenotype‡	No.
Midnight	Parent 1	*CyvCyv*	PveIF4E[1](**C**) PveIF4E[1] (**C**)	18	Susceptible	18
Black Knight	Parent 2	*cyvcyv*	PveIF4E[4](**A**) PveIF4E[4] (**A**)	18	Resistant	18
Midnight x Black Knight	F_1	*Cyvcyv*	PveIF4E[1](**C**) PveIF4E[4] (**A**)	18	Susceptible	18
Midnight x Black Knight	F_2	*cyvcyv*	PveIF4E[4](**A**) PveIF4E[4] (**A**)	49	Resistant	49
		Cyvcyv	PveIF4E[1](**C**) PveIF4E[4] (**A**)	97	Susceptible	144
		CyvCyv	PveIF4E[1](**C**) PveIF4E[1] (**C**)	47		

† Expected genotypic ratio for F_2 population of 1 AA: 2 AC: 1 CC; $\chi^2 = 0.046$, $P = 0.977$ ($df = 2$).
‡ Expected phenotypic ratio for F_2 population of 1 Resistant: 3 Susceptible; $\chi^2 = 0.015$, $P = 0.902$ ($df = 1$).

Table 2.6. Cosegregation of KASPar SNP PveIF4E[1,3,4] (**A**) allele with systemic necrosis and KASPar SNP PveIF4E[2] (**G**) allele with resistance to NL 3 D as conditioned by *bc-3* in the Black Knight x Raven populations

			PveIF4E[1,3,4]__PveIF4E[2]			
Genotype	Population	NL3 D Resistance	SNP (**A**332**G**)	No.†	Phenotype‡	No.
Black Knight	Parent 1	*cyvcyv*	PveIF4E[1,3,4] (**A**) PveIF4E[1,3,4] (**A**)	18	SN	18
Raven	Parent 2	*bc-3bc-3*	PveIF4E[2] (**G**) PveIF4E[2] (**G**)	18	Resistant	18
Black Knight x Raven	F_1	*cyv bc-3*	PveIF4E[1,3,4] (**A**) PveIF4E[2] (**G**)	18	SN	18
Black Knight x Raven	F_2	*bc-3bc-3*	PveIF4E[2] (**G**) PveIF4E[2] (**G**)	50	Resistant	50
		cyv bc-3	PveIF4E[1,3,4] (**A**) PveIF4E[2] (**G**)	84	SN	134
		cyvcyv	PveIF4E[1,3,4] (**A**) PveIF4E[1,3,4] (**A**)	50		

† Expected genotypic ratio for F_2 population of 1 AA: 2 AG: 1 GG; $\chi^2 = 1.392$, $P = 0.498$ ($df = 2$).
‡ Expected phenotypic ratio for F_2 population of 1 Resistant: 3 Susceptible; $\chi^2 = 0.464$, $P = 0.496$ ($df = 1$); 'SN' denotes 'systemic necrosis'.

Table 2.7. Allelic discrimination of KASPar SNP PveIF4E^{3} (**A**) and PveIF4E^{2} (**G**) alleles in the Clipper x B/R RIL105-25 populations

Genotype	Population	ClYVV Resistance	PveIF4E^{1,3,4}__PveIF4E^{2} SNP (**A**332**G**)	No. †	Phenotype	No.
Clipper	Parent 1	*cyvcyv*	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**A**)	18	Resistant	18
B/R RIL 105-25	Parent 2	*bc-3bc-3*	PveIF4E^{2} (**G**) PveIF4E^{2} (**G**)	18	Resistant	18
Clipper x B/R RIL 105-25	F_1	*cyvbc-3*	PveIF4E^{1,3,4} (**A**) PveIF4E^{2} (**G**)	18	Resistant	18
Clipper x B/R RIL 105-25	F_2	*cyvcyv*	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**A**)	32	Resistant	132
		cyvbc-3	PveIF4E^{1,3,4} (**A**) PveIF4E^{1,3,4} (**G**)	65		
		bc-3bc-3	PveIF4E^{2} (**G**) PveIF4E^{2} (**G**)	35		

† Expected genotypic ratio for F_2 population of 1 AA: 2 AG: 1 GG; $\chi^2 = 0.166$, $P = 0.921$ ($df = 2$).

Table 2.8. *PveIF4E* haplotype analysis as enabled by the three KASPar assays employed in the research.

Genotype	ClYVV† Resistance	*PveIF4E* Allele	KASPar SNP Assays: PveIF4E[1]__PveIF4E[2,3,4] SNP (**C**227**A**)	KASPar SNP Assays: PveIF4E[1,4]__PveIF4E[2,3] SNP (**C**159**A**)	KASPar SNP Assays: PveIF4E[1,3,4]__PveIF4E[2] SNP (**A**332**G**)
Dubbele Witte	S	*PveIF4E[1]*	PveIF4E[1] (**C**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Midnight	S	*PveIF4E[1]*	PveIF4E[1] (**C**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Hystyle	S	*PveIF4E[1]*	PveIF4E[1] (**C**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
GN 1140	*cyv*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Black Knight	*cyv*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Jolanda	*desc*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Amanda	*cyv*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Baby Bop	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Laureat	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Paloma	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Polder	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Sonesta	*r*	*PveIF4E[4]*	PveIF4E[2,3,4] (**A**)	PveIF4E[1,4] (**C**)	PveIF4E[1,3,4] (**A**)
Imuna	*cyv, desc*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
Evolutie	*desc*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
Clipper	*cyv*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
CY-10 S_4	*cyv*	*PveIF4E[3]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[1,3,4] (**A**)
IVT 7214	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
Raven	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
B/R RIL105-25	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
USWK-6	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)
USWKH x H S_4	*bc-3*	*PveIF4E[2]*	PveIF4E[2,3,4] (**A**)	PveIF4E[2,3] (**A**)	PveIF4E[2] (**G**)

† 'S' denotes 'susceptible', 'r' denotes 'resistant'; confirmed by screening 5 to 10 plants of each cultivar in three independent experiments, with 'r' confirmed by ELISA.

2.4 DISCUSSION

The germplasm evaluation (Table 2.1), the allelism testing (Table 2.3), the complete association between unique nonsynonymous SNPs in *PveIF4E* alleles and resistance to ClYVV and / or NL 3 D (Table 2.4), and the KASPar allele-specific assays (Tables 2.5, 2.6, 2.7, 2.8) all contribute to the evidence that *cyv*, *desc*, and *bc-3* comprise a series of recessive resistance alleles at the *Bc-3* locus. This allelic series conditions strain and species-specific resistance spectra to the potyviruses ClYVV, BCMV, and BCMNV in common bean. In light of this, the nomenclature for the *Bc-3* locus should be revised in accordance with the guidelines for gene nomenclature set forth by the Bean Improvement Cooperative (BIC) (Porch, 2012).

The first genetic symbol designated to an allele at this locus was *by-3*, identified in GN1140 (Provvidenti and Shroeder, 1973), although it was subsequently revised to *cyv* (Provvidenti, 1987). It was previously demonstrated that *desc* from Jolanda was allelic with the ClYVV resistance present in the cultivar Imuna [previously reported as *cyv* (Park and Tu, 1991; Sato et al., 2003)]. We also demonstrate that the resistance from Jolanda is allelic with that possessed by Clipper and Amanda (previously reported as *cyv* by Tu, 1983, and Park and Tu, 1991, respectively). The assignment of the independent gene status and symbol *desc* was not warranted, nor does it appear to have ever been formally accepted by the BIC (Porch, 2009). As the *bc-3* gene symbol, first identified in IVT 7214 (Drijfhout, 1978) was assigned prior to the designation of *cyv*, it retains priority as the gene symbol for the locus. The original *bc-3* allele from IVT 7214 retains its designation as *bc-3*, and it conditions the widest spectrum of resistance to BCMV and BCMNV when in

combination with *bc-u* or *I* (Kelly et al., 1995). The *cyv* allele first reported in GN 1140 should be designated *bc-3*^{2} (Table 2.9) to reflect its recessive resistance to ClYVV-NY, its differential susceptibility to NL 3 D, and the order in which it was discovered.

Table 2.9. Revised gene symbol nomenclature for the *Bc-3* locus

Genotype	Accession #	ClYVV Resistance	*PveIF4E* allele †	Gene Symbol
Dubbele Witte	PI 549695	Susceptible	*PveIF4E*^{1}	*Bc-3*
IVT 7214	PI 602987	*bc-3*	*PveIF4E*^{2}	*bc-3*
Clipper	PI 278776	*cyv*	*PveIF4E*^{3}	
GN 1140	PI 549667	*cyv*	*PveIF4E*^{4}	*bc-3*^{2} ‡
Jolanda	G 7591	*desc*		

† In the absence of direct functional complementation, *PveIF4E* remains as the candidate gene for *Bc-3*.
‡ Proposed here and under review by the Bean Improvement Cooperative Genetics Committee.

A causative relationship between mutations in PveIF4E and *bc-3* resistance is probable based on the clear precedent of recessive potyvirus resistance conferred by amino acid substitutions in eIF4E. This can be the result of as few as one amino acid substitution, or in many cases, an allelic series of eIF4E is present where various subtle signature amino acid substitutions in the surface loops of the eIF4E protein confer unique strain and species specific resistance spectra [(the *pvr2* alleles in *Capsicum annuum* (Charron et al., 2008; Kang et al., 2005b; Ruffel et al., 2002), the *pot-1* allele in *Solanum lycopersicum* (Ruffel et al., 2005), the *mo1* allele in *Lactuca sativa* (Nicaise et al., 2003), the *sbm 1* alleles *in Pisum sativum* (Andrade et al., 2009; Bruun-Rasmussen et al., 2007; Gao et al., 2004), the *rym4* and *rym5* alleles in *Hordeum vulgare* (Hofinger et al., 2011; Stein et al., 2005) and the *zym*-FL allele in *Citrullus lanatus* (Ling et al., 2009)]. The pattern of predicted amino acid substitutions

in the surface loops of PveIF4E and the complete association with differential resistance presented here closely resembles the numerous other pathosystems characterized. In addition, there is close alignment of mutations in PveIF4E with mutations in eIF4E that confer potyvirus resistance alleles present in the plant species *C. annuum* (Kang et al., 2005b; Ruffel et al., 2002), *L. sativa* (Nicaise et al., 2003), and *P. sativum* (Bruun-Rasmussen et al., 2007; Gao et al., 2004) (Naderpour et al., 2010). Here we provide strong evidence for an association between a single amino acid substitution at position 76 (A76E) and resistance to ClYVV-NY, and for a single amino acid substitution at position 111 (D111G) and differential resistance to NL 3 D.

This result parallels previous research that has revealed a co-evolutionary 'arms race' where positive Darwinian selection has acted on single amino acid sites of eIF4E to create recessive virus resistance genes as the result of coevolution with the virus, where the viral-encoded VPg functions as the pathogenicity determinant, and is also under positive selection (Cavatorta et al., 2008; Charron et al., 2008; Moury et al., 2004). Statistical methods for inferring positive selection in combination with the *a priori* data available from eIF4E resistance alleles in *C. annuum* (Charron et al., 2008), *S. lycopersicum* (Ruffel et al., 2005), and *P. sativum* (Gao et al., 2004) demonstrated high precision and power to positively identify the single amino acid sites involved in potyvirus resistance (Cavatorta et al., 2008). Remarkably, out of the ten amino acid positions examined, positions 76 and 110 were identified with the highest posterior probabilities (Cavatorta et al., 2008). In *C. annuum*, *P. sativum*, and *P. vulgaris*, resistance to a potyvirus is either confirmed, or associated with the substitution of an alanine amino acid residue at position 76 with either an aspartic residue (*C. annuum*,

P. sativum), proline residue (*P. sativum*), or a glutamic acid residue (A76E) (*P. vulgaris*). The D111G amino acid substitution in PveIF4E^2, and associated with differential resistance to NL 3 D, is also remarkably close to position 110. Previous research has revealed that the pathogenicity determinant of the *cyv* resistance breaking strain (ClYVV-Br) maps to the central region of the VPg and is likely responsible for the restoration of pathogenicity (Sato et al., 2003).

Direct functional conferral of resistance, and restoration of susceptibility is warranted, but common bean has lacked efficient transformation capabilities. A *Bean pod mottle virus* (BPMV) (family *Comoviridae*, genus *Comovirus*)-based virus induced gene-silencing (VIGS) vector for common bean has recently been developed (Diaz-Camino et al., 2011) and may prove to be a useful tool for confirming the function of PveIF4E in potyvirus resistance in future research. Functional analysis of PveIF4E-potyvirus VPg interactions through protein-protein interaction experiments may also be warranted to better characterize, understand, and predict resistance spectra.

Establishment of the allelic relationships between the known sources of resistance to ClYVV in common bean directly informs the definition of the target genotype for the most effective resistance, at least in terms of the strains known in the United States. The possibility of pyramiding the previously identified putative independent genes is no longer possible, but it is clear that the original *bc-3* allele from IVT7214 conditions the widest spectrum of resistance. The *bc-3* allele is the only allele that conditioned resistance to all strains of ClYVV, including ClYVV-WI and ClYVV-OR, except when in the IVT 7214 background (Larsen et al., 2008), whereas

bc-3^2 (present in Jolanda and Imuna) did not. It is difficult to speculate as to why IVT 7214 may be the exception, as it possesses the *bc-u* allele that should condition the full expression of *bc-3* (Drijfhout, 1978). In addition, the genotype USCR-8 that possesses *bc-3* in the absence of both the *I* gene and *bc-u* (Miklas and Hang, 1998), was resistant to ClYVV-WI and ClYVV-OR (Larsen et al. 2008). It is still somewhat unclear whether *bc-3* or *bc-3*^{2} are effective for resistance to ClYVV in the absence of *bc*-u and/or *I* because all of the genotypes investigated in this research possessed one, or both genes. Another exception is GN 1140, as while it possesses *bc-3*^{2}, and is mildly susceptible to NL 3 D, it was resistant to ClYVV-WI and ClYVV-OR (Larsen et al., 2008). The genotype GN UI 31 may be an analogous case as it was the source of two recessive genes that conditioned resistance to the BYMV-S strain from Oregon (Tatchell et al., 1985), so it is feasible that GN 1140 and GN UI 31 possess an additional resistance gene, perhaps an alternate allele of *bc-u* (Strausbaugh et al., 2003) that widen their strain specific resistance spectrum to include ClYVV-WI and ClYVV-OR where other *bc-3*^{2} genotypes do not (Larsen et al., 2005a). GN UI 31 has been demonstrated to have mixed reactions to specific BCMV/BCMNV strains potentially due to admixture or residual heterozygosity in previous research (Forster et al., 1994; Miklas et al., 2000). Additional effort is needed to obtain a more thorough characterization of the spectrum of resistance to ClYVV and BCMV/BCMNV pathogenicity groups provided by *bc-3*^{2} alone, and in combination with *bc-u* and/or *I*.

The three KASPar assays developed here provide a suite of rapid, user-friendly, putatively functional molecular markers that have the capability to detect and differentiate all four of the *PveIF4E* alleles characterized thus far. These assays are an

improvement over the RsaI CAPS marker associated with *bc-3* resistance (Naderpour et al., 2010) because in addition to the *PveIF4E*^{2} allele, the RsaI site at position 193 of the coding sequence is also present in the *PveIF4E*^{3} allele (Appendix 2.2; 2.3; 2.4), which is not associated with resistance to NL 3 D. The PveIF4E KASPar assays are also more convenient to employ in marker-assisted selection (MAS) because they do not require enzymatic digestion or gel electrophoresis, however they do require fluorescent endpoint detection capabilities. The RsaI CAPS marker therefore remains useful when this capability is absent, but the resistance to NL 3 D of the donor parent must be known or confirmed. The discrimination capability of the KASPar assays developed here may also have the potential for rapid characterization of germplasm through the detection of mutant alleles, particularly for detecting predicted amino acid substitutions at positions 76 and 111. Table 2.8 summarizes the employment of the three assays for allelic discrimination in the 21 common bean genotypes studied and simultaneously provides a guide on how to use the KASPar assays for allelic discrimination and MAS.

This research contributes to an enhanced understanding of the gene-for-gene relationships between potyviruses and resistance alleles associated with mutations in *PveIF4E* at the *Bc-3* locus in common bean. This knowledge combined with the KASPar assays, continue in the development of a platform to elucidate the role of eIF4E-mediated recessive resistance in common bean. Advanced research in this area in other crop plants has been rewarding, with new insights into the biology (e.g. complementation interactions with mutations in eIF(iso)4E for an enhanced resistance spectrum (Hwang et al., 2009; Rubio et al., 2009; Ruffel et al., 2006)), the

demonstration of new tools for rapid identification of novel alleles by allele mining [e.g. High-resolution melting analysis (HRM) (Hofinger et al., 2009; Hofinger et al., 2011)], and to even create novel resistance alleles by mutagenesis (Piron et al., 2010). The research presented here provides a foundation on which to advance similar research in common bean. The KASPar assays developed here should also assist in the utilization of *bc-3* resistance important to common bean growing regions around the world.

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CHAPTER 3

RESISTANCE TO *Clover yellow vein virus* IN COMMON BEAN

3.1 INTRODUCTION

Clover yellow vein virus (ClYVV) (family *Potyviridae*, genus *Potyvirus*) causes disease and crop damage that may reduce yields of common bean (*Phaseolus vulgaris* L.) production wherever the crop is grown, and particularly in temperate regions (Barnett et al., 1987; CABI, 2000; Crnov and Gilbertson, 2001; Dizadji and Shahraeen, 2011; Larsen and Myers, 2006; Ortiz et al., 2009; Provvidenti and Shroeder, 1973; Sasaya et al., 1997; Tu, 1988). ClYVV was previously considered a 'severe' (Provvidenti and Shroeder, 1973) or 'necrotic' (Tu, 1983) strain of its close relative *Bean yellow mosaic virus* (BYMV) because the two viruses share many properties including overlapping host range, serological cross-reaction (Bos et al., 1977; Jones and Diachun, 1977; Sasaya et al., 1997), and because in some hosts, including common bean, infection results in similar symptoms (Larsen et al., 2008; Provvidenti and Morales, 2005a). ClYVV has since been delimited as a distinct species from BYMV based on sequencing and alignment of coat proteins and 3' non-coding regions from multiple isolates of both viruses (Uyeda et al., 1991; Tracy et al., 1992).

ClYVV induces severe symptoms that include pronounced stunting, prominent yellow mosaic, leaf distortion, systemic necrosis of the phloem and apex, and in some cases premature death of the infected plant (Provvidenti and Morales, 2005b). Although it appears that no data is available on the impact of ClYVV on yield in

common bean, these symptoms can make ClYVV a potentially devastating pathogen. Of particular concern is the possibility for certain strains, or certain interactions, to induce mottling, twisting, malformation, and interior necrosis of bean pods (Larsen and Myers, 2006; Larsen et al., 2008; Provvidenti and Morales, 2005b). Because the snap bean market class is harvested as fresh succulent immature pods, those pods that are deformed and/or necrotic are rejected and discarded by wholesalers and processors. In regions where snap beans are produced for processing, an epidemic of ClYVV and the presence of deformed and/or necrotic pods above a threshold may result in total yield loss and/or rejection of the harvest for entire production fields. The potential for this scenario to occur in the Great Lakes Region of the United States where the farm-gate value of processing snap bean exceeds $140 million (USDA-NASS, 2012) has increased markedly in recent years.

Though both ClYVV and BYMV have been reported from this region in the past (Dickson and Natti, 1968; Provvidenti and Shroeder, 1973) their incidence has increased in frequency as part of an emerging complex of aphid-transmitted viruses (Larsen et al., 2002; Larsen et al., 2008; Nault et al., 2004; Shah et al., 2006; Tolin and Langham, 2010) that have been associated with the accidental introduction of the soybean aphid (*Aphis glycines* Matsumara) to the United States (Hill et al., 2001; Ragsdale, 2004). While it is unknown whether *A. glycines* can transmit ClYVV, the virus is transmitted in a nonpersistent manner by numerous noncolonizing aphid species that are present in the region (Nault, 1997; Provvidenti and Morales, 2005b; Shah et al., 2006). When inoculum is available and noncolonizing aphid populations are high, the transmission of ClYVV and other viruses can occur rapidly as plants are

probed briefly and the aphids continue their dispersal (Nault et al., 2004). Given this situation, and the ineffectiveness of insecticides to control aphid vectors and reduce the incidence of virus infection in beans and other crops (Madden et al., 2000; Nault et al., 2004), deployment of host plant resistance to ClYVV is the most effective strategy to minimize crop damage and enhance the stability of snap bean production in the region.

Natural genetic variation for resistance to ClYVV is available in the primary gene pool of common bean (reviewed by Hart and Griffiths, 2013). Resistance is conditioned by the *bc-3* (Larsen et al., 2008) and *bc-3*^{2} alleles and has been demonstrated to be closely associated with non-synonymous single nucleotide polymorphisms (SNPs) in *P. vulgaris* eukaryotic translation initiation factor 4E (*PveIF4E*) (Hart and Griffiths, 2013). With the exception of functional analysis, there is strong evidence that suggests that the predicted amino acid substitution that results from the C227A non-synonymous SNP in *PveIF4E* is the determinant of resistance for ClYVV (Hart and Griffiths, 2013). There is similar evidence that the determinant for resistance to the 'NL 3D' strain of *Bean common mosaic necrosis virus* (BCMNV), and by extension, most known strains of BCMNV and several strains of *Bean common mosaic virus* (BCMV) (Miklas et al., 1998), results from the predicted amino acid substitution conferred by the A332G non-synonymous SNP (Hart and Griffiths, 2013). Kompetitive Allele Specific PCR (KASP) assays were developed to detect and select for these non-synonymous SNPs that are the putative functional determinants of resistance to ClYVV, BCMV, and BCMNV (Hart and Griffiths, 2013). These non-synonymous SNPs cosegregated with their predicted resistance or susceptibility

phenotype in three separate segregating populations, and in a panel of 21 common bean genotypes. Although these KASP assays have been suggested for germplasm exploration or allele mining in common bean germplasm collections they have not been employed to do so.

The objective of this research was to further characterize ClYVV resistance in common bean through the phenotypic evaluation of 391 accessions of the common bean core collection, an expanded collection of 99 snap bean cultivars and breeding lines, and an extended host differential panel of 75 genotypes with known resistance and/or susceptibility to strains of ClYVV, BYMV, BCMV, BCMNV, and *Bean golden yellow mosaic virus* (BGYMV). The objective of assembling and evaluating an extended host differential panel containing multiple resistance alleles to other viruses was to examine whether their spectrum of resistance included ClYVV, and to determine whether there was a correlation between resistance to ClYVV and resistance to other viruses. The goal was to simultaneously obtain a better understanding of ClYVV resistance in common bean and to potentially identify new sources of resistance and/or novel resistance alleles. The phenotypic evaluation allowed for a more in-depth evaluation of the relationship between non-synonymous SNPs in *PveIF4E* and potyvirus resistance in a much larger panel of germplasm than was previously employed (Hart and Griffiths, 2013). It also served as an opportunity to further validate the previously designed KASP assays for future resistance allele mining in germplasm collections.

3.2 MATERIALS AND METHODS

3.2.1 Plant material, virus strain, and inoculation

Seed of 391 accessions (Central America: 93, Mexico: 213, South America: 85, Not available: 31) of the *P. vulgaris* core collection was obtained from the USDA-ARS Western Regional Plant Introduction Station, Pullman WA (W6) (Appendix 3.1). To screen for resistance in the snap bean market class, seed of 99 snap bean cultivars and breeding lines was assembled from the W6 and from a collection at Cornell University New York State Agricultural Experiment Station (NYSAES), Geneva, NY (Appendix 3.2). The core collection accessions and snap bean cultivars that were identified as resistant through an initial rapid screen were combined with additional common bean cultivars and breeding lines with known differential virus resistance genes and/or responses to viral pathogens to assemble an extended host differential panel for further evaluation. The 75 common bean genotypes of the extended host differential panel (Table 3.1) were selected to provide multiple representatives of each of the 12 BCMV/BCMNV host resistance groups characterized to date, as well as additional common bean genotypes known to possess resistance alleles to other viruses, namely *bgm-1* and *bgm-2* resistance to BGYMV (Velez et al., 1998; Blair and Morales, 2008), *bc-3*^{2} resistance to ClYVV (Hart and Griffiths, 2013), and *By-2* resistance to BYMV (Dickson and Natti, 1968). The extended host differential panel was comprised of seed from the W6, USDA-ARS, NYSAES, Michigan State University, and CIAT collections.

The germplasm was evaluated for resistance to the ‘New York’ strain of

Table 3.1. Accession, designation, seed class, gene pool, BCMV/BCMNV host group, allele at *I* locus present, recessive virus resistance alleles present, reference cited for recessive virus resistance alleles, *PveIF4E* allele present, *Bc-3* allele present, and phenotypic response to ClYVV-NY in an extended host- differential panel of 75 genotypes of *Phaseolus vulgaris*.

Accession†	Designation‡	Seed class§	Gene pool¶	HG#	*I*††	Recessive Resistance‡‡	Ref§§	*PveIF4E* allele¶¶	*Bc-3* ##	ClYVV-NY †††
PI 377736	Dubbele Witte	Snap	Sn	0	*i*	none	1	1	*Bc-3*	S‡‡‡
PI 598999	Str. Grn. Ref.	Snap	Sn	1	*i*	*bc-u*	1	1	*Bc-3*	S
PI 599003	Imuna	Snap	Sn$^{?}$	2	*i*	*bc-u, bc-1*	2	3	*bc-3*^{2}	R‡‡‡
PI 599000	Red. GrnLf. C	Snap	Sn	2	*i*	*bc-u, bc-1*	1	1	*Bc-3*	S
PI 550035	Agate	Pinto	MA	3	*i*	*bc-u, bc-1*^{2}	3	4	*bc-3*^{2}	R
PI 599004	Red. GrnLf. B	Snap	Sn	3	*i*	*bc-u, bc-1*^{2}	1	1	*Bc-3*	S
PI 615391	Emerson	GN	MA	?	?	*bc-u, bc-1*^{2}	3	1	*Bc-3*	S
PI 599006	GN UI 123	GN	MA	3	*i*	*bc-u, bc-1*^{2}	1	4	*bc-3*^{2}	R
PI 549667	GN 1140	GN	MA	3	*i*	*bc-u, bc-1*^{2}	2	4	*bc-3*^{2}	R‡‡‡
PI 578262	Harold	Pink	MA	3	*i*	*bc-u, bc-1*^{2}	3	5	*Bc-3*	S
PI 550055	Harris	GN	MA	3	*i*	*bc-u, bc-1*^{2}	3	5	*Bc-3*	S
PI 550039	Ivory	GN	MA	3	*i*	*bc-u, bc-1*^{2}	3	5	*Bc-3*	S
PI 550014	NW-59	Small Red	MA	3	*i*	*bc-u, bc-1*^{2}	3	1	*Bc-3*	S
PI 550016	NW-63	Small Red	MA	3	*i*	*bc-u, bc-1*^{2}	3	1	*Bc-3*	S
PI 550028	Ouray	Pinto	MA	3	*i*	*bc-u, bc-1*^{2}	3	4	*bc-3*^{2}	R
PI 550138	Sapphire	GN	MA	3	*i*	*bc-u, bc-1*^{2}	3	4	*bc-3*^{2}	R
PI 550278	Topaz	Pinto	MA	3	*i*	*bc-u, bc-1*^{2}	3	4	*bc-3*^{2}	R
PI 554602	UI 537	Pink	MA	3	*i*	*bc-u, bc-1*^{2}	3	4	*bc-3*^{2}	R
PI 578261	Victor	Pink	MA	3	*i*	*bc-u, bc-1*^{2}	3	5	*Bc-3*	S

Continued on next page.

Table 3.1. Continued

Accession†	Designation‡	Seed class§	Gene pool¶	HG#	*I*††	Recessive Resistance‡‡	Ref§§	*PveIF4E* allele¶¶	*Bc-3* ##	ClYVV-NY †††
PI 549940	Viva	Pink	MA	3	*i*	*bc-u, bc-1*^{2}	3	1	*Bc-3*	S
PI 599009	Michelite 62	Navy	MA	4	*i*	*bc-u, bc-2*	1	1	*Bc-3*	S
PI 550013	NW-410	Pinto	MA	4	*i*	*bc-u, bc-2*	3	4	*bc-3*^{2}	R
PI 549695	Sanilac	Navy	MA	4	*i*	*bc-u, bc-2*	1	1	*Bc-3*	S
PI 599014	Pinto UI 114-8	Pinto	MA	5	*i*	*bc-u, bc-1, bc-2*	1	4	*bc-3*^{2}	R
PI 550010	Pindak	Pinto	MA	?	?	*bc-u, bc-1*^{2}*, bc-2*	3	5	*Bc-3*	S
PI 550041	Spinel	GN	MA	?	*i*	*bc-u, bc-1*^{2}*, bc-2*	3	4	*bc-3*^{2}	R
PI 599031	UI 129	Pinto	MA	?	*i*	*bc-u, bc-1*^{2}*, bc-2*	1	4	*bc-3*^{2}	R
PI 599015	UI 31 GN	GN	MA	6	*i*	*bc-u, bc-1*^{2} *bc-2*^{2*}	1	4	*bc-3*^{2}	R
PI 550129	Fiesta	Pinto	MA	6	*i*	*bc-u, bc-2*^{2}	3	5	*Bc-3*	NLL(SN)
PI 550038	Garnet	Small Red	MA	6	*i*	*bc-u, bc-2*^{2}	3	4	*bc-3*^{2}	R
PI 549937	Gloria	Pink	MA	6	*i*	*bc-u, bc-2*^{2}	3	4	*bc-3*^{2}	R
PI 599016	Monroe	Navy	MA	6	*i*	*bc-u, bc-2*^{2}	1	1	*Bc-3*	NLL(SN)
PI 599018	RedMex.UI 35	Small Red	MA	6	*i*	*bc-u, bc-2*^{2}	1	1	*Bc-3*	NLL(SN)
W6 27753	Yolano	Pink	MA	6	*i*	*bc-u, bc-2*^{2}	3	4	*bc-3*^{2}	R
PI 602987	IVT 7214	Snap	Sn	7	*i*	*bc-u, bc-2, bc-3*	1	2	*bc-3*	R‡‡‡
W6 36148	Don Timoteo	Black	MA	7	*i*	*bc-u, bc-3*	4	2	*bc-3*	R
ARS	USCR-8	Cranberry	AN^{MA}	?	*i*	*bc-3*	5	2	*bc-3*	R
PI 599021	Black Turtle 1	Black	MA	8	*I*	none	1	1	*Bc-3*	S
PI 550032	Midnight	Black	MA	8	*I*	none	2	1	*Bc-3*	S‡‡‡
PI 550288	Hystyle	Snap	Sn	?	*I*	?	1	1	*Bc-3*	S‡‡‡
W6 42706	Evolutie	Snap	Sn$^?$	9	*I*	*bc-1, bc-3*^{2}	2	3	*bc-3*^{2}	R‡‡‡

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Table 3.1. Continued

Accession†	Designation‡	Seed class§	Gene pool¶	HG#	*I*††	Recessive Resistance‡‡	Ref§§	*PveIF4E* allele¶¶	*Bc-3* ##	ClYVV-NY †††
G 7591	Jolanda	Snap	$Sn^{?}$	9	*I*	*bc-1*	2	4	*bc-3*^{2}	R‡‡‡
PI 599026	Amanda	Snap	$Sn^{?}$	10	*I*	*bc-1*^{2}, *bc-3*^{2}	1	4	*bc-3*^{2}	R‡‡‡
PI 599030	92-US-1006	Pinto	MA	11	*I*	*bc-u, bc-2*^{2}	1	5	*Bc-3*	NLL(SN)
PI 599029	IVT 7233	Snap	Sn^{MA}	11	*I*	*bc-u, bc-1*^{2}, *bc-2*^{2}	1	1	*Bc-3*	NLL(SN)
MSU	B/R RIL105-25	Navy	MA	12	*I*	*bc-3*	2	2	*bc-3*	R‡‡‡
PI 594325	TARS-VR-1S	Navy	MA	12	*I*	*bc-3*	6	2	*bc-3*	R
MSU	Raven	Black	MA	12	*I*	*bc-3*	7	2	*bc-3*	R‡‡‡
PI 618814	USDK-4	Kidney	AN^{MA}	12	*I*	*bc-3*	8	2	*bc-3*	R
PI 602998	USLK-2	Kidney	AN^{MA}	12	*I*	*bc-3*	8	2	*bc-3*	R
PI 618815	USWK-6	W. Kidney	AN^{MA}	12	*I*	*bc-3*	8	2	*bc-3*	R‡‡‡
CU	USWKH x H S_4	Snap	AN^{MA}	12	*I*	*bc-3*	2	2	*bc-3*	R‡‡‡
PI 181954	Homs No. 14	Landrace	ND	12	*I*	*bc-3*	9	5	*Bc-3*	S§§§
PI 642144	Baby Bop	Snap	$Sn^{?}$	?	*I*	?, *bc-3*^{2}	2	4	*bc-3*^{2}	R‡‡‡
CU	Black Knight	Black	MA	?	*I*	?, *bc-3*^{2}	2	4	*bc-3*^{2}	R‡‡‡
PI 278776	Clipper	Navy	MA	?	*i*	?, *bc-3*^{2}	2	3	*bc-3*^{2}	R‡‡‡
CU	CY-10 S_4	Snap	Sn^{MA}	?	*I*	?, *bc-3*^{2}	2	3	*bc-3*^{2}	R‡‡‡
PI 550261	Laureat	Snap	$Sn^{?}$	?	*I*	?, *bc-3*^{2}	2	4	*bc-3*^{2}	R‡‡‡
CU	Paloma	Snap	$Sn^{?}$	?	*I*	?, *bc-3*^{2}	2	4	*bc-3*^{2}	R‡‡‡
CU	Polder	Snap	$Sn^{?}$	?	*I*	?, *bc-3*^{2}	2	4	*bc-3*^{2}	R‡‡‡
ARS	R31 27	EL	MA	?	?	?	10	3	*bc-3*^{2}	R
W6 28061	RH13	Snap	Sn^{MA}	?	*I*	?	11	4	*bc-3*^{2}	R
PI 549738	Scout	Pinto	MA	?	?	?	12	4	*bc-3*^{2}	R

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Table 3.1. Continued

Accession†	Designation‡	Seed class§	Gene pool¶	HG#	*I*††	Recessive Resistance‡‡	Ref§§	*PveIF4E* allele¶¶	*Bc-3* ##	ClYVV-NY †††
CU	Sonesta	Snap (wax)	$Sn^{?}$	?	*I*	?, *bc-3*^{2}	2	4	*bc-3*^{2}	R‡‡‡
PI 151407	Sangre de Toro	Landrace	MA	?	*i*	?	-	3	*bc-3*^{2}	R
PI 634536	Amadeus 77	Small Red	MA	?	*I*	?, *bgm-1*	13	5	*Bc-3*	S
PI 639174	Carrizalito	Small Red	MA	?	*I*	?, *bgm-1*	14	1	*Bc-3*	S
PI 613168	GMR 1	Black	MA	?	*I*	?, *bgm-1*	15	1	*Bc-3*	S
PI 606249	Morales	White	MA	?	*I*	?, *bgm-1*	16	1	*Bc-3*	S
CU	Scorpio	Snap	Sn^{MA}	?	*I*	?, *bgm-1*	17	1	*Bc-3*	S
PI 595892	Tio Canela-75	Small Red	MA	?	*I*	?, *bgm-1*	18	5	*Bc-3*	S
PI 634536	Amadeus 77	Small Red	MA	?	*I*	?, *bgm-1*	13	5	*Bc-3*	S
PI 639174	Carrizalito	Small Red	MA	?	*I*	?, *bgm-1*	14	1	*Bc-3*	S
PI 613168	GMR 1	Black	MA	?	*I*	?, *bgm-1*	15	1	*Bc-3*	S
PI 606249	Morales	White	MA	?	*I*	?, *bgm-1*	16	1	*Bc-3*	S
CU	Scorpio	Snap	Sn^{MA}	?	*I*	?, *bgm-1*	17	1	*Bc-3*	S
PI 595892	Tio Canela-75	Small Red	MA	?	*I*	?, *bgm-1*	18	5	*Bc-3*	S
PI 606251	PR9443-4	Kidney	AN^{MA}	?	*I*	?, *bgm-2*	19	1	*Bc-3*	S
CU	B-21	Black	MA	?	*I*	none	20	1	*Bc-3*	R(SN)
CU	B28S2C	Snap	Sn	?	*I*	none	-	1	*Bc-3*	R(SN)
CU	SP 17B	GN	MA	?	*I*	*bc-3*^{2}	21	4	*bc-3*^{2}	R

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Table 3.1. Continued

† Accession classification; PI and W6 = USDA; G = CIAT; CU = Cornell University; MSU = Michigan State University; ARS = USDA-ARS
‡ Designation abbreviations; Str. Grn. Ref. = Stringless Green Refugee; RedGrnLf = Redlands Green Leaf; RedMex. = Red Mexican.
§ Seed class abbreviations; GN = Great Northern; EL = Experimental line; LR = Landrace accession.
¶ Gene pool classification and origin of ClYVV-NY resistance; Sn = Snap bean; $Sn^{?}$ = Snap bean, resistance source unknown; MA = Middle American; Sn^{MA} = Snap bean, resistance source Middle American; AN^{MA} = Andean, resistance source Middle American; ND = Not determined.
# BCMV/BCMNV Host group (HG) classification based on *I* allele and recessive resistance alleles present; ? = Not determined.
†† Genotypes assigned based on references cited (Ref)§§; *I* = *I* allele present; *i* = *I* allele absent; ? = Not determined
‡‡ Recessive resistance genotypes assigned based on references cited (Ref)§§; ? = Not determined.
§§ References for *I* and recessive resistance alleles; Appendix 3.3.
¶¶ *PveIF4E* allele assigned based on KASPar allele-specific genotyping according to Table 3.2.
Bc-3 allele assigned according to Table 3.2.
††† ClYVV-NY response; S = susceptible; R = resistant; NLL(SN) = heterogeneous reaction where all plants' primary leaves exhibited necrotic local lesions (NLL), and some plants later exhibited systemic necrosis and plant death (SN); R(SN) = heterogeneous reaction where all plants were initially classified as resistant (R), and some plants later exhibited systemic necrosis and plant death (SN).
‡‡‡ *PveIF4E* allele and response to ClYVV-NY previously reported (Hart and Griffiths, 2013).
§§§ PI 181954; reported as the source of *bc-3* in IVT 7214 (Drijfhout, 1978), 30 plants were susceptible and homozygous for the *PveIF4E* susceptibility allele.

ClYVV (ClYVV-NY), obtained from the Rosario Provvidenti collection at the NYSAES (Provvidenti and Schroeder, 1973), and further characterized by recent research (Larsen et al., 2008; Hart and Griffiths, 2013). ClYVV-NY was used for the resistance evaluation because in previous research it has identified the resistance phenotype conditioned by either the *bc-3* or *bc-3*^{2} resistance alleles reliably and thus far without exception (Hart and Griffiths, 2013). ClYVV-NY has been stored long-term as frozen (-80°C) and as desiccated tissue, and was multiplied for the experiments by periodical transfer (~3 weeks) to newly expanded primary leaves (7-10 days after planting) of the susceptible snap bean cultivar Hystyle.

The methodology for greenhouse based mechanical inoculation, resistance evaluation, and virus detection of ClYVV-NY was performed as reported in previous research (Hart and Griffiths, 2013). Briefly, in order to rapidly screen the *P. vulgaris* core collection and the snap bean cultivars, 14 seeds of each accession and cultivar were divided and planted into nine, 10- by 10-cm cells of 18-cell flats (Speedling Inc.) that contained Cornell mix (Boodley and Sheldrake, 1972) and then thinned so that nine plants each of two accessions or cultivars occupied each flat. The extended host differential panel of common bean genotypes with known differential resistance genes and/or responses to viral pathogens, as well as any core collection accessions or snap bean cultivars that were symptomless after the initial rapid screen in 18-cell flats (Table 3.1) were planted in 10, 14.6- by 14.6-cm pots and evaluated in two additional separate experiments. All plant materials were grown in greenhouses at the NYSAES where temperature was maintained at 24°C day/21°C night, and with supplemental lighting to maintain a 14 h photoperiod. Routine watering, fertilization, and integrated

pest management regimes were applied.

In both container formats, eight plants of each genotype were inoculated at the VC stage (Brick, 2005) when the primary leaves were fully expanded (7-10 days after planting), leaving one or two plants as uninoculated controls. Inoculum was prepared by homogenizing symptomatic, recently expanded ClYVV-NY infected trifoliate leaves (1:10 w/v) of Hystyle in cold 0.01 M phosphate buffer (pH 7.0) with a mortar and pestle. Inoculum was applied with the pestle by gently rubbing the homogenate onto primary leaves that had been dusted with silicon carbide powder (23µm, [Electron Microscopy Sciences]). Inoculated plants were rinsed with deionized water immediately after inoculation. Plants of the extended host differential panel that did not exhibit symptoms of virus infection 10 days post the first inoculation (dpi) were inoculated again to prevent escape.

3.2.2 Evaluation of resistance to ClYVV

Inoculated plants were monitored daily for symptoms of ClYVV infection. In the rapid 18-cell flat format, core collection accessions and snap bean cultivars were classified as either resistant or susceptible based on visual symptoms at 12 dpi. Relative to uninoculated controls, susceptible plants exhibited severe stunting often accompanied by a reddish-brown necrosis in the veins of the inoculated primary leaves which resulted in either top necrosis and plant death, or a delay in development that failed to produce a fully expanded first trifoliate leaf within the time period of evaluation. Resistant plants remained symptomless and development was identical to the uninoculated controls. Resistance to ClYVV was confirmed through entry into the

extended host differential panel (Table 3.1) and the two subsequent resistance evaluations of that panel. Resistance to ClYVV-NY was evaluated similarly for the extended host differential panel, except that visual symptoms were assessed and plants were classified as either resistant or susceptible at 10, 22, 30, 45, 60, and 90 dpi. Resistance was further confirmed by subjecting tissue samples to enzyme-linked immunosorbent assay (ELISA). The polyclonal antibody test system for the ELISA was ClYVV-C81 & Pratt (AC Diagnostics Inc.), and was performed according to the manufacturers instructions. Absorbance at OD_{405nm} was measured with a multi-mode microplate reader (Synergy 2, Biotek Instruments Inc.) following final incubation and after two additional one-hour intervals. Only absorbance reads greater than or equal to two times that of the healthy negative control were considered to be positive for presence of the virus.

3.2.2 Allele-specific genotyping of *PveIF4E*

The 75 common bean genotypes of the extended host differential panel (Table 3.1) were genotyped with three 'PveIF4E' KASP SNP assays (LGC Genomics) (Table 3.2). The primer sequences of these assays, and the methods for DNA isolation and genotyping were carried out according to previously published protocols (Hart and Griffiths, 2013). Briefly, genomic DNA was isolated from ~50 mg (1 cm^2) of tissue from newly emerged trifoliate leaves with a standard protocol for common bean (Afanador et al., 1993). DNA was quantified by using Quant-iT PicoGreen (Life Technologies) and a multi-mode microplate reader according to the manufacturer's instructions. The KASP assays were performed in 8 μL reaction volumes containing

20 ng of DNA, 4 µL of 2x KASP reaction mix, and 0.11 µL of primer assay mix, and PCR amplification was performed as specified (Robinson and Holmes, 2011) in a Gradient Master Cycler instrument (Eppendorf). Post-PCR allele-specific fluorescence was acquired with the ViiA7 Real-Time PCR system (Life Technologies) and allele calls were made with the 'genotyping experiment' module of the ViiA7 software. Allele calls were checked manually, and in some cases were converted from uncalled to called based on the similarity of their data with other known alleles. The PveIF4E KASP assay IDs, the target SNPs, and their associated *Bc-3* allele were assigned according to Table 3.2.

Table 3.2. KASP SNP assays to determine *PveIF4E* alleles and predict the associated *Bc-3* allele and resistance spectrum.

KASP assay ID (SNP) †				
PveIF4E^{1}__PveIF4E^{2,3,4}	PveIF4E^{1,4}__PveIF4E^{2,3}	PveIF4E^{1,3,4}__PveIF4E^{2}		
(C227A)	(C159A)	(A332G)	*PveIF4E* allele‡	*Bc-3* allele
C	C	A	*PveIF4E*^{1} (1)	*Bc-3*
A	A	G	*PveIF4E*^{2} (2)	*bc-3*
A	A	A	*PveIF4E*^{3} (3)	*bc-3*^{2}
A	C	A	*PveIF4E*^{4} (4)	*bc-3*^{2}
C	A	A	*PveIF4E*^{5} (5)	*Bc-3*

† KASPar primer sequences published previously (Hart and Griffiths, 2013).
‡ *PveIF4E* allele; number in parentheses (1-5) corresponds with Table 3.1.

3.3 RESULTS AND DISCUSSION

3.3.1 Resistance to ClYVV in the USDA-ARS core collection

The rapid phenotypic screen of 391 accessions of the USDA-ARS *P. vulgaris* Core Collection identified a single accession (PI 151407) that lacked symptoms of ClYVV infection in all plants. This accession was confirmed to be ClYVV resistant by

visual symptoms and by negative ELISA results when it was re-evaluated as part of the extended host differential panel (Table 3.1). The resistance possessed by PI 151407 was further characterized as described in the 'Allele-Specific Genotyping of *PveIF4E*' section below. The overwhelming frequency of susceptibility in the core collection is not entirely surprising given that most of the 30,000-plus accessions of the CIAT common bean collection do not possess any resistance alleles to BCMV (Morales, 2006). Although ClYVV is a distinct potyvirus species from BCMV, resistance to ClYVV is conditioned by recessive alleles at the BCMV/BCMNV *Bc-3* resistance locus, notably the *bc-3* allele (Larsen et al., 2008; Hart and Griffiths, 2013). The *bc-3* allele in combination with either the *I* and/or *bc-u* allele(s) provides resistance to all known strains of BCMV and BCMNV worldwide (Drijfhout 1978; Kelly et al., 1995), except the recombinant NL 3 K strain of BCMV/BCMNV (Larsen et al., 2005), and therefore it would have been reliably identified in the evaluation of the CIAT collection in accessions where it was present.

3.3.2 Resistance to ClYVV in snap beans

The rapid phenotypic screen of 99 snap bean cultivars identified one additional snap bean breeding line, RH13, and confirmed the ClYVV resistance of 10 snap bean cultivars and two breeding lines (Appendix 3.2) that failed to produce symptoms in response to infection with ClYVV-NY. These snap bean genotypes remained symptomless and ELISA-negative when re-evaluated as part of the extended host differential panel (Table 3.1; Appendix 3.2). The snap bean breeding line RH13 was released in 1977 by the French National Institute for Agricultural Research (INRA)

Versailles, France, and was selected to possess a genetic factor donated by GN UI 123 that conditioned resistance to ClYVV (previously typed as BYMV) (Fouilloux and Bannerot, 1977). Five of the snap bean cultivars (Baby Bop, Laureat, Paloma, Polder, Sonesta) with resistance to ClYVV-NY were also selected to produce marketable pods of small-sieve size that in some cases may be referred to as small sieve, or 'whole-pack' snap beans depending on their intended use. Given that these cultivars were released from one to three decades later than RH13, and that small-sieve cultivars predominate U.S. whole-pack processing, European, and particularly French snap bean production, we hypothesized that RH13 may have been the donor of the GN UI 123 allele for ClYVV resistance in these cultivars. We further investigated this possibility as described in the 'Allele-Specific Genotyping of *PveIF4E*' section below. Although data appears unavailable on the incidence and impact of ClYVV infection in snap beans in Europe, resistance to ClYVV (previously typed as BYMV strains) has been an important selection criterion in at least some breeding programs (Walkey and Innes, 1978; Walkey et al., 1983). The snap bean cultivars identified here are valuable both as sources of *bc-3*^{2} resistance to ClYVV, and possibly for immediate deployment as potential replacements for susceptible small-sieve snap bean cultivars in production regions affected by ClYVV epidemics.

3.3.3 Resistance to ClYVV in an extended host differential panel

The evaluation of the 75 common bean genotypes of the extended host differential panel (Table 3.1) identified resistance to ClYVV in the majority of the major market classes of common bean. ClYVV-resistant genotypes were identified in

the Snap bean, Pinto, Great Northern, Pink, Small Red, Navy, Black, and Kidney dry bean market classes (Table 3.1). Many of these genotypes are held in public collections and are therefore available and accessible as donors of ClYVV resistance to bean breeding programs around the world.

The BCMV/BCMNV host group classification of the genotypes and their respective alleles at the *I* and *Bc-* loci have been determined by meticulous research efforts over several decades since they were first delimited (Drijfhout et al., 1978), and the genotype assignments at these loci in Table 3.1 were acquired according to the references indicated in Table 3.1 and in Appendix 3.3. The host groups were demonstrated to be of variable predictive value for resistance to ClYVV. Host groups 0, 1, and 8 were predictably susceptible based on previous research (Larsen et al., 2008; Hart and Griffiths, 2013). The predictive value of host groups 5, 9, and 10 where all genotypes that were tested were resistant to ClYVV due to the ubiquitous presence of *bc-3*^{2} could not be ascertained with confidence as too few genotypes were tested. Given that alleles of *Bc-3* are inherited independently from alleles of *I* and the other *Bc-* loci (Drijfhout, 1978; Johnson et al., 1997; Kelly et al., 2003, Miklas et al., 2006), it is likely that common bean genotypes of these host groups exist that do not possess *bc-3*^{2}. Resistant and susceptible genotypes were identified in host groups 2, 3, and 4, making these host group designations alone of no predictive value for ClYVV resistance. The ClYVV resistance present in these host groups was conditioned by the *bc-3*^{2} allele as confirmed by *PveIF4E* allele-specific genotyping (Table 3.1). In contrast, host groups 6 and 11 that possess the *bc-u*, *bc-2*^{2} allele combination, and host groups 7, 12, and one undesignated host group that possess the *i, bc-u*, *bc-3*, the *I, Bc-*

u, bc-3, and the *i, Bc-u*, *bc-3* allele combinations respectively, exhibited responses that can be predicted based on these host genotypes.

Bean genotypes of host groups 6 and 11 that possess the *i, bc-u*, *bc-2*^{2}, *Bc-3* and *I, bc-u*, *bc-2*^{2}, *Bc-3* allele combinations respectively, exhibited necrotic local lesions on their primary leaves at 10 dpi. This response was previously reported in the cultivar Monroe when it was identified as an effective local lesion host for ClYVV (Dwadash-Shreni and Stavely, 1984), although no additional *bc-u*, *bc-2*^{2} genotypes were tested. The results presented in Table 3.1 demonstrate this response exclusively in four additional *bc-u*, *bc-2*^{2}, *Bc-3* genotypes (Fiesta, Red Mexican UI 35, 92-US-1006, and IVT 7233) and establish that this response to ClYVV is conditioned by the *bc-u*, *bc-2*^{2} allele combination. ELISA did not detect the presence of ClYVV in the primary leaves that exhibited necrotic local lesions or in the trifoliate leaves of IVT7233 (data not shown). This result confirms that a hypersensitive ClYVV-resistance phenotype is conferred by the *bc-u*, *bc-2*^{2} allele combination. Interestingly, approximately three or four out of the eight inoculated plants of each of these five genotypes in each experiment exhibited symptoms of systemic vein and top necrosis approximately 20-30 dpi and these plants ultimately died. Given that all of the *bc-u*, *bc-2*^{2}, *Bc-3* cultivars responded similarly, where some individuals exhibited systemic necrosis and other individuals continued normal development through physiological maturity, we hypothesized that the heterogeneous reaction was likely not due to genetic segregation. Rather, we hypothesized that the response was due to either the emergence of a resistance-breaking isolate of ClYVV-NY or a specific interaction with other experimental variables that initiated an uncontrolled systemic phloem

necrosis. Though this hypothesis requires further research to confirm, ClYVV has been demonstrated to overcome *bc-3*^{2} (previously designated *desc*) resistance in common bean (Sato et al., 2003). Although in this case it was not a hypersensitive resistance being overcome as in the case of the *bc-u*, *bc-2*^{2} allele combination discussed here, resistance-breaking isolates have been documented in almost all cases of hypersensitive resistance to viruses (Harrison, 2002).

Host groups 7, 12, and one undesignated host group that possessed the *i, bc-u*, *bc-3*, the *I, Bc-u, bc-3,* and the *i, Bc-u*, *bc-3* allele combinations respectively were predictably resistant to ClYVV as demonstrated by previous research (Larsen et al. 2008; Hart and Griffiths, 2013). The resistance spectrum conditioned by the *bc-3* allele alone is not as broad as when in combination with the *I* and/or *bc-u* alleles as the *i*, *Bc-u*, *bc-3* genotype conditions resistance to all known strains of BCMNV, but not all strains of BCMV (US1, US3, US7) (Miklas et al., 1998). The breeding line USCR-8 (Miklas and Hang, 1998) was the only *i*, *Bc-u*, *bc-3* genotype tested for resistance to ClYVV. Given that USCR-8 was determined to be resistant to ClYVV-NY here and to the presumably more virulent Wisconsin strain of ClYVV (ClYVV-WI) (Larsen and Miklas, 2008) suggests that the *bc-3* allele may be able to act independently to condition resistance to ClYVV. This hypothesis needs to be tested more thoroughly as it is possible that another uncharacterized gene that interacts with *bc-3* may be involved (Miklas et al., 1998). The landrace accession PI 181954 that was reported to be the original source and donor of the *bc-3* allele (Dickson and Natti, 1966; Drijfhout, 1978) was susceptible to ClYVV-NY in this evaluation. The results of both the phenotypic resistance evaluation and of allele-specific genotyping of *PveIF4E*

confirmed that all 30 plants that were tested of this accession did not possess the *bc-3* allele in the homozygous, nor heterozygous state. We were unable to verify this accession as the original donor of *bc-3*.

Additional common bean genotypes that are not part of the BCMV/BCMNV host groups were also included in the extended host differential panel (Table 3.1). The genotypes that were known to possess the *bc-3*^{2} allele (Hart and Griffiths, 2013) were included to serve as resistant controls, to monitor the potential for resistance breakdown, and to further illustrate the diverse market classes in which ClYVV-resistance is present. There was no evidence of resistance breakdown in any of these resistant controls over the entire course of both evaluations. Bean genotypes known to possess the BGYMV resistance alleles *bgm-1* or *bgm-2* (Velez et al. 1998; Blair and Morales, 2008) were included to investigate whether those recessive alleles' resistance spectra also included ClYVV. All genotypes that possessed *bgm-1* or *bgm-2* genotypes were susceptible to ClYVV-NY.

Finally, three genotypes that possess the *By-2* allele for resistance to BYMV (Dickson and Natti, 1968) were included in the panel to investigate the resistance spectrum of this allele, and because of past confusion regarding the taxonomy of ClYVV and BYMV (Bos et al., 1977; Tracy et al. 1992). B-21 is nearly isogenic with Black Turtle-1, except that it possesses the *By-2* allele introgressed from an unknown *P. coccineus* accession (Dickson and Natti, 1968; Provvidenti et al. 1989). B28S2C is a NYSAES snap bean breeding line that possesses the *By-2* allele introgressed from B-21. SP 17B is a multiple virus resistant breeding line that possesses both *By-2* and *bc-3*^{2} (previously designated *cyv*) (Scully et al. 1995). The genotypes B-21 and B28S2C

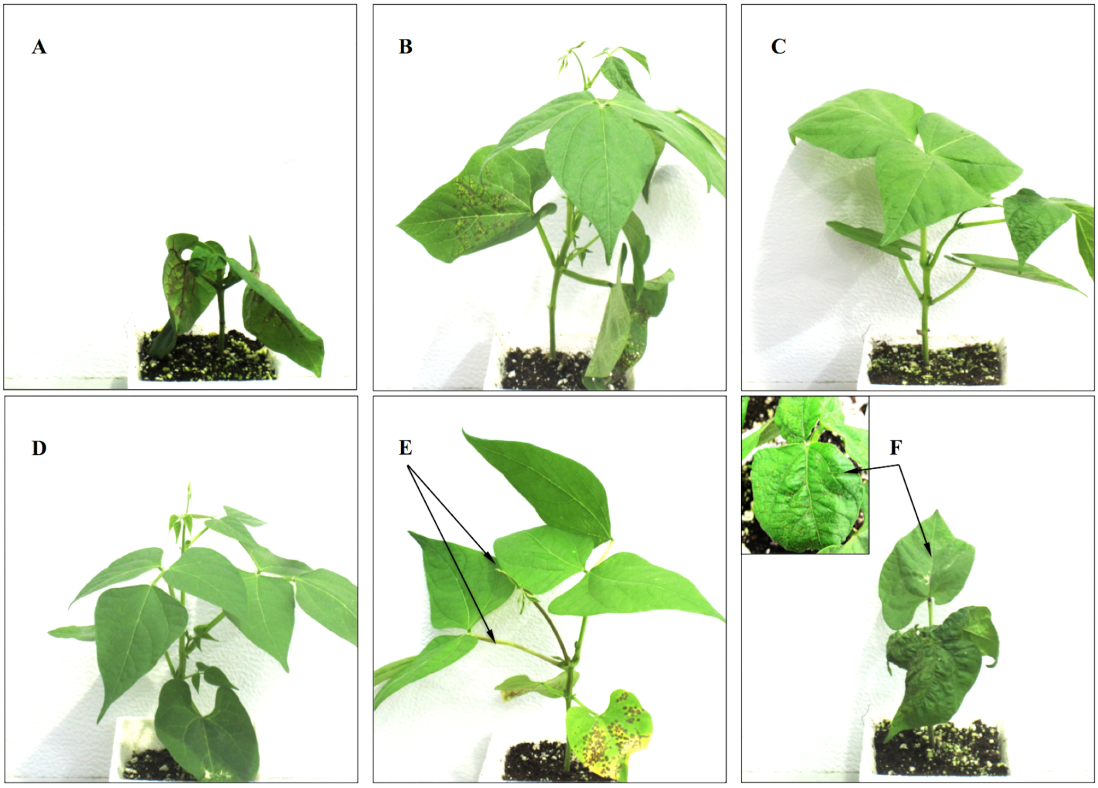
that both possessed the *I, By-2*, *Bc-3* combination exhibited a heterogeneous response, where all plants were initially symptomless, and ClYVV could not be detected by ELISA or RT-PCR (data not shown) in the inoculated primary leaves, until approximately 10-20 dpi when approximately half of the plants began to display necrosis in the secondary veins of trifoliate leaves, and eventually systemic phloem necrosis and plant death. This result strongly suggests that *By-2* provides resistance to ClYVV, but that it may also be subject to resistance breaking variants of ClYVV, or that it may also be under the influence of other experimental variables that could include host genetic background, inoculum concentration and/or timing of inoculation, and environmental conditions. Additional research is needed to test these hypotheses. SP17B with the *I*, *By-2*, *bc-3*^{2} genotype remained resistant due to the presence of *bc-3*^{2}. Examples of all of the ClYVV interaction phenotypes described herein are illustrated in Figure 3.1.

3.3.4 Allele-specific genotyping of *PveIF4E*

The results of the allele-specific genotyping of *PveIF4E* in the extended host differential panel are presented in Table 3.1. Given that the *bc-3* and the *bc-3*^{2} resistance alleles condition different resistance spectra, but that both condition resistance to ClYVV, the allele-specific genotyping allowed for the unambiguous assignment of the allele present at three key positions in the *PveIF4E* coding sequence, and subsequently for the assignment of the correct phenotypic resistance allele according to Table 3.2. A fifth *PveIF4E* allele (*PveIF4E*^{5}) with a unique pattern of non-synonymous SNPs in the coding sequence was identified. Though this allele is

novel, the non-synonymous SNP at position 159 is not associated with resistance to ClYVV. The hypothesis that the snap bean breeding line RH13 may be the donor of the GN UI 123 ClYVV resistance allele to other snap beans was supported by the presence of the same allele (*PveIF4E*^{4}) in GN UI 123 and all of the snap beans with unknown parentage (excluding Cornell snap bean breeding lines). Although 21 of the 75 genotypes were genotyped with these KASP assays in previous research (Hart and Griffiths, 2013), the complete association between the various *PveIF4E* alleles with resistance and susceptibility to ClYVV in 54 additional genotypes held. These results further validate the potential use of these markers that are based on the putative causal polymorphisms for large scale high-throughput allele mining of *P. vulgaris* germplasm.

Figure 3.1. *Clover yellow vein virus* (ClYVV)-common bean interaction phenotypes. A) Severe stunting and reddish-brown necrosis in veins of the primary leaves of BT-1 and other susceptible genotypes. B) Necrotic local lesion response on primary leaves of IVT 7233 and other genotypes that possess the *bc-u*, *bc-2*^{2} allele combination. C) Resistance response of B-21 and other genotypes that possess the *By-2* allele. D) Resistance response of UI 537 and other genotypes that possess *bc-3*^{2} or *bc-3*. E) Systemic necrosis response heterogeneous amongst individual plants of IVT 7233 and other genotypes that possess *bc-u*, *bc-2*^{2}. F) Systemic necrosis in secondary veins, systemic necrosis and top death in B-21 and other genotypes that possess *By-2* in the absence of *bc-3*^{2}.



The allele-specific genotyping of the host differential panel also revealed that the *PveIF4E* alleles associated with resistance were detected exclusively in genotypes that are classified as members of the Middle American gene pool, or in other genotypes where Middle American donors contributed the resistance. This pattern of

origin for virus resistance alleles may extend to the resistance alleles at other loci, and may even be specifically traceable to Middle American genotypes of landrace Durango, as accessions of this landrace have previously been noted to possess resistance to numerous viruses including *Bean dwarf mosaic virus* (BDMV), *Bean golden mosaic virus* (BGMV), BGYMV, BCMV, BCMNV, BYMV, and *Cucumber mosaic virus* (CMV) (Morales, 2006). The only landrace accession from the *P. vulgaris* core collection that was identified as resistant to ClYVV was PI 151047, and which possessed *bc-3*^{2} (*PveIF4E*^{4}), has also been associated with landrace Durango (McClean et al., 2012). Targeted screening for a wide range of virus resistance in wild germplasm from the central plateau of Mexico and of landrace Durango may be warranted. In addition, an in-depth investigation into the phylogenetic origins of *PveIF4E* alleles is warranted to provide greater insight into the evolution of this important source of potyvirus resistance in common bean.

3.4 CONCLUSIONS

The *P. vulgaris* core collection (Appendix 3.1), the snap bean cultivars (Appendix 3.2), and the extended host differential panel (Table 3.1) represent the widest range of germplasm evaluated for ClYVV resistance to date. Taken together the results synthesize a new model for ClYVV resistance in common bean. This model of resistance should now include the *bc-u*, *bc-2*^{2} allele combination (Drijfhout 1978; Dwadash-Shreni and Stavely, 1984) and *By-2* (Dickson and Natti, 1968) in addition to *bc-3* (Larsen et al. 2008) and *bc-3*^{2} (Hart and Griffiths, 2013). Although neither *bc-2*^{2} nor *By-2* have been located on the core genetic map of common bean (Miklas et al.,

2006), there is evidence that they are alleles at independent loci (for *bc-u*, *bc-2*^{2}, *bc-3* see Drijfhout, 1978; for *By-2* see Scully et al. 1995). The characterization of these additional ClYVV resistance alleles and the putative independence of these four loci now offers the opportunity to pyramid these alleles to enhance the potential for more durable resistance to ClYVV.

Achieving the *bc-u*, *bc-2*^{2}, *bc-3*, *By-2* allele combination will require additional research and the development of robust molecular markers due to the relationships that exist amongst these alleles. In terms of resistance to ClYVV, the *bc-3* and *bc-3*^{2} alleles are epistatic to the *bc-u*, *bc-2*^{2} allele combination in that they mask the necrotic local lesion response conditioned by *bc-u*, *bc-2*^{2} (Kelly et al., 1995). The complete lack of symptoms conditioned by *By-2* (in the absence of necrosis) would also presumably be epistatic to the necrotic local lesions produced by the *bc-u*, *bc-2*^{2} allele combination, but this hypothesis needs to be tested. A dominant and recessive epistatic interaction exists between *bc-3* alleles and *By-2* for ClYVV resistance, and molecular markers would be essential to pyramid these genes. Otherwise, progeny testing homozygous recessive *bc-3* genotypes (identified with BCMNV NL 3D or KASP SNP assays) with BYMV can identify the presence of *By-2* (Scully et al. 1995).

The research presented here provides a new understanding of the genetics of resistance to ClYVV. This broadened understanding requires additional research into the inheritance and molecular basis for virus-resistance allele interactions. Most importantly, this research has identified a wide range of publicly available sources of resistance to ClYVV in most of the major market classes of common bean, and has further demonstrated and validated a suite of KASP SNP assays to speed the

introgression and pyramiding of ClYVV resistance into breeding programs.

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CHAPTER 4

GENOTYPING-BY-SEQUENCING (GBS) ENABLED MAPPING AND MARKER DEVELOPMENT FOR THE *By-2* POTYVIRUS RESISTANCE ALLELE IN COMMON BEAN

4.1 INTRODUCTION

An aphid-transmitted virus disease complex has emerged as a major cause of crop damage and economic loss to snap bean (*Phaseolus vulgaris* L.) production in the Great Lakes Region of the United States (Larsen et al., 2002; Larsen et al., 2008; Nault et al., 2004; Shah et al., 2006). This disease complex is particularly threatening because snap beans can generate in excess of US $185 million per year in farm-gate value for the eight states that comprise this region (USDA-NASS, 2013). The increased frequency of epidemics coincided with the accidental introduction of the soybean aphid (*Aphis glycines* Matsumura) to the United States in 2000 (Hill et al., 2001; Ragsdale et al., 2004), even though soybean aphids were not the dominant aphid species detected in snap bean fields sampled during virus disease epidemics in New York State in 2002 and 2003 (Shah et al., 2006). Attempts to control aphid vectors as a means to reduce the nonpersistent transmission and spread of the prevalent viruses is generally considered ineffective (Nault et al., 2004; Pedersen et al., 2007; Perring et al., 1999; Raccah, 1986). In this situation, control measures need to be preventative, and the most compatible and effective preventative strategy is to plant cultivars with resistance to the prevalent viruses of the complex. The absence of commercially acceptable cultivars with resistance to the prevalent viruses leaves the snap bean crop

and the associated processing industry vulnerable to continued periodic yield losses.

Bean yellow mosaic virus (BYMV) (family *Potyviridae*, genus *Potyvirus*) is one of the prevalent viruses of this virus disease complex (Shah et al., 2006; Tolin and Langham, 2010). BYMV is a monopartite, positive sense RNA virus that is distributed throughout the world, has a broad host range, and is capable of causing economic losses in a range of monocotyledonous and dicotyledonous plant families (Wylie et al., 2008). In the United States, BYMV was first detected in common bean in 1948 in western Oregon (Crumb and McWhorter, 1948) and has been observed in important common bean production regions across the country (Tolin and Langham, 2010). As part of recent epidemics in the Great Lakes Region, BYMV was detected in 2002 and 2003 in 79% of surveyed snap bean fields in New York State (Shah et al., 2006). Although within field incidence ranged widely, the incidence reached as high as 43% of the plants sampled in one field (Shah et al., 2006). Estimates of the impact of BYMV infection on the yield and quality of snap beans are unavailable, but the symptoms of BYMV infection in snap beans are generally severe when infection occurs early in development. The symptoms of BYMV infection in snap bean depend on the cultivar, virus strain, environment, and the plant developmental stage at infection, but the responses to infection include prominent mosaic and distortion of leaves, a reduction in plant vigor and stunting, delayed maturity, and slight malformation and mottling of the pods (Provvidenti and Morales, 2005).

Several sources of resistance to BYMV have been identified in previous decades, but biological differences in the various virus strains employed and the subsequent taxonomical revision of some of those strains could lead to confusion. At

least three distinct 'strains' of BYMV were previously used to study the inheritance of resistance in common bean. The BYMV isolates that were referred to as the 'pod-distorting' strain (Grogan and Walker, 1948), the 'severe' strain (Provvidenti and Shroeder, 1973; Tatchell et al., 1985), and the 'necrotic' strain (Tu, 1983) were later revised to be isolates of *Clover yellow vein virus* (ClYVV), a related but distinct virus species from BYMV (Tracy et al., 1992). Resistance to these strains was conditioned by a single recessive allele first designated *by-3* from GN 1140 (Provvidenti and Schroeder, 1973), or two complementary recessive alleles from UI 31 GN (Tatchell et al., 1985). The *by-3* gene symbol was subsequently revised to *cyv* to reflect this change, although this allele has been examined in detail recently and the symbol revised to *bc-3*^{2} to reflect its status as an allele at the potyvirus resistance locus *Bc-3* (Hart and Griffiths, 2013).

The second distinct strain of BYMV known as 'pea virus 2 (PV2),' which has also been referred to in the past as *Pea mosaic virus* (PMV), and more recently as the 'pea strain' of BYMV (BYMV-P) (Provvidenti and Morales, 2005) was considered distinct because it did not infect any common bean genotypes, including those that were susceptible to other strains of BYMV (Schroeder and Provvidenti, 1966). This strain was eventually discovered to infect the cultivar Scotia and Black Turtle Soup, and a single dominant gene designated *By* from Red Kidney was demonstrated to condition resistance (Schroeder and Provvidenti, 1968). It appears that the vast majority of common bean genotypes possess resistance to this strain (Provvidenti and Morales, 2005).

The third distinct 'strain' of BYMV studied in common bean is the type strain

of the virus, and this taxon also encompasses considerable genetic diversity worldwide (Wylie et al., 2008). In terms of common bean, isolates of the type strain of BYMV are capable of infecting the widest range of host genotypes, including those that possess resistance to ClYVV (Baggett et al., 1966; Dellavalle et al., 1994). Three complimentary recessive alleles donated by UI 31 GN were reported to condition resistance to the type strain of BYMV (Bagget and Frazier, 1957; Tatchell et al., 1985), but it appears that this resistance has never been further characterized or introgressed into additional market classes.

The *By-2* allele is the only BYMV resistance allele that has been further characterized and introgressed into common bean market classes, and was recently introgressed into the snap bean market class as part of the research presented here. *By-2* was donated by an unnamed accession of *P. coccineus* in an interspecific cross with a Blue Lake pole snap bean to develop the experimental line BL-6 (Dickson and Natti, 1968), although Kelvedon Marvel was later reported as a *P. coccineus* source of BYMV and multiple virus resistance by the same authors (Provvidenti and Dickson, 1981). *By-2* conditioned resistance in this population was demonstrated to segregate according to a Mendelian ratio for a single dominant gene (Dickson and Natti, 1968). BL-6 was subsequently used as the donor of the *By-2* allele in an initial cross with Black Turtle-1 to develop the near-isogenic BYMV-resistant dry black bean breeding line B-21 after six backcrosses to Black Turtle-1 (Provvidenti et al., 1989). *By-2* was also introgressed into breeding lines of other dry bean market classes (Scully et al., 1990a; Scully et al., 1990b; Scully et al., 1995), but was never introgressed into the snap bean market class beyond the development of BL-6. Unfortunately this line was

never deployed and appears to no longer be available.

Recent occurrences of BYMV epidemics as part of the aphid-transmitted virus disease complex of snap bean in the United States have renewed our interest in the characterization, evaluation, and potential deployment of resistance conditioned by the *By-2* allele. In order to introgress and pyramid this allele with other virus resistance alleles into elite backgrounds in an efficient manner, the map location and linkage relationships with other economically important alleles needs to be established, and codominant molecular marker-assisted selection of the *By-2* allele needs to be enabled. *P. vulgaris* however, has lacked a highly efficient and cost-effective platform for single nucleotide polymorphism (SNP) discovery and genotyping for use in genomics-assisted crop improvement until very recently (Hyten et al., 2010).

This important constraint has been further lifted due to the recent release of the first chromosome scale assembly of a high quality reference genome for common bean (*Phaseolus vulgaris* v1.0) (DOE-JGI and USDA-NIFA, 2013) and the development and refinement of genotyping-by-sequencing (GBS) (Elshire et al. 2011). GBS is a robust platform capable of simultaneously discovering and genotyping high numbers of SNPs in multiplexed barcoded sequencing libraries in plant species with or without a reference genome (Elshire et al. 2011). The technique's flexibility is based on the reduction of genome complexity through restriction enzyme digestion, and efficient, user-friendly, multiplexed reduced representation library construction. The technique has been demonstrated successfully for a wide range of population genetic investigations in a rapidly growing number of crop plants [Barba et al., 2013 (*Vitis* spp.); Chen et al., 2013 (*Pinus contorta* and *Picea glauca*); Elshire et al., 2011 (*Zea*

mays); Lu et al., 2013 (*Panicum virgatum*); Ly et al., 2013 (*Manihot esculenta*); Morris et al., 2013 (*Sorghum bicolor*); Poland et al., 2012 (*Triticum aestivum*, *Horedum vulgarre*); Spindel et al., 2013 (*Oryza sativa*); Ward et al., 2012 (*Rubus idaeus*)]. The only immediate needs for adapting GBS to a new species are to empirically select an appropriate restriction enzyme for complexity reduction and to determine the appropriate concentrations of adapter barcode concentrations for the fragments produced by the restriction digestion.

The objectives of this research were to locate the physical region of the common bean genome that contained the *By-2* allele, and then to develop and validate allele-specific molecular markers for marker-assisted selection of *By-2* conditioned resistance to BYMV. In order to accomplish this objective, we sought a better understanding of the inheritance of the resistance response conditioned by *By-2* and simultaneously developed a collection of useful genetic materials for mapping. To survey the molecular genetic variation that segregated in this germplasm, we sought to empirically adapt GBS to discover and genotype genome-wide SNPs. Here we describe our effort to utilize GBS coupled with the common bean genome and a novel genome-wide association study (GWAS) approach to identify highly significant SNPs, convert them into allele-specific molecular markers, and to validate and enable rapid and cost effective genomics-assisted breeding for major effect alleles in common bean.

4.2 MATERIALS AND METHODS

4.2.1 Germplasm, populations, and DNA isolation

A series of recombinant inbred lines (RILs) were generated as a result of two cycles of line development to introgress the *By-2* allele from the black bean donor line B-21 (Provvidenti et al., 1989) into the processing snap bean background of the cultivar Hystyle. An F_6:F_8 experimental line designated B28S2C was the result of pedigree and progeny selection for the absence of lethal alleles (Hannah et al., 2007), resistance to BYMV, snap bean pod and seed traits, and plant architecture throughout six inbreeding generations following a three-way cross between B-21 and Hystyle, and the flat-podded snap bean cultivar Tapia. B28S2C was then backcrossed to Hystyle to recover additional recurrent parent genome and to develop BC_1F_1, and BC_1F_2 populations. Single plants from the BC_1F_2 and subsequent $BC_1F_{2:3}$ families were selected for snap bean pod and seed traits and plant architecture. Selected individuals were screened for resistance to BYMV as $BC_1F_{3:4}$ lines. Single plants from resistant, susceptible, and segregating $BC_1F_{3:4}$ lines were selected and selfed, and a subset of six $BC_1F_{4:5}$ BYMV resistant lines and six $BC_1F_{4:5}$ BYMV susceptible lines were selected for progeny testing. The 12 $BC_1F_{4:5}$ differential lines were tested for BYMV resistance and seven plants from five homozygous resistant lines, one heterozygous line, and six homozygous susceptible lines were selected, sampled for DNA extraction, and allowed to self pollinate. The 84 $BC_1F_{5:6}$ RILs were progeny tested with BYMV to confirm their genotypes and phenotypes. These 84 lines were also progeny tested with ClYVV to confirm that *By-2* also conditioned resistance to this virus (Table 4.1).

In addition to the 84 $BC_1F_{5:6}$ differential RILs, the four parental genotypes

Hystyle, B-21, Tapia, and Black Turtle-1 were included as controls in both the phenotyping and genotyping experiments (Table 4.1). Black Turtle-1 was included as it was the susceptible recurrent parent in six backcrosses to introgress *By-2* into the black bean market class that resulted in the development of B-21 (Provvidenti et al., 1989). Black Turtle-1 and B-21 were considered as near isogenic lines (NILs) and they were expected to segregate only for alleles in the immediate region of the *By-2* locus.

Table 4.1. Plant materials phenotyped for resistance to BYMV-NY and ClYVV-NY, their respective allele at the *By-2* locus, the number of plants from each $BC_1F_{4:5}$ recombinant inbred line (RIL) selected for DNA isolation and genotyping-by-sequencing (GBS), and the number of $BC_1F_{5:6}$ individuals that exhibited delayed systemic necrosis (dSN) when progeny tested with BYMV-NY.

Accession†	Designation	Phenotype‡	Genotype	DNA§	$F_{5:6}$ w/ dSN¶
PI 550288	Hystyle	Susceptible	*by-2*	3	-
CU	Tapia	Susceptible	*by-2*	3	-
PI 599021	Black Turtle-1	Susceptible	*by-2*	3	-
PI 557487	B-21	Resistant	*By-2*	3	-
CU	F5R-1	Resistant	*By-2*	7	4/60
CU	F5R-2	Resistant	*By-2*	7	10/45
CU	F5R-3	Resistant	*By-2*	7	18/62
CU	F5R-4	Resistant	*By-2*	7	3/63
CU	F5R-5	Resistant	*By-2*	7	3/61
CU	F5R-6	Resistant	*By-2*	7	4/56
CU	F5S-1	Susceptible	*by-2*	7	0
CU	F5S-2	Susceptible	*by-2*	7	0
CU	F5S-3	Susceptible	*by-2*	7	0
CU	F5S-4	Susceptible	*by-2*	7	0
CU	F5S-5	Susceptible	*by-2*	7	0
CU	F5S-6	Susceptible	*by-2*	7	0

† Accessions classified as PI are from the USDA Western Regional Plant Introduction Station and those classified as CU are from Cornell University.

‡ Phenotypes confirmed by progeny tests with BYMV-NY and ClYVV-NY

§ The number of individual plants from each cultivar or breeding line that was sampled for DNA extraction, assigned an *Ape*KI barcode (see Supplemental Table S3), and genotyped with GBS.

¶ The number of $BC_1F_{5:6}$ plants that exhibited delayed systemic necrosis (dSN) out of the total number of plants tested. Approximately nine plants were progeny tested for each of the seven lines selected for GBS.

Three F_2 populations were developed to study the inheritance of resistance conditioned by *By-2* and for marker-cosegregation analysis. B28S2C was crossed to Hystyle to develop two separate F_1 and F_2 populations of 20 and ~200 individuals respectively. The first F_2 population of 200 individuals was used to study the inheritance of *By-2*. The second population of 20-F_1 and 185-F_2 individuals was used for both inheritance and marker-cosegregation analysis. All F_1 plants of these two populations were confirmed to be heterozygous based on plant architecture and pod traits. We also crossed B-21 to BT-1 to generate 10 F_1 and 167 F_2 (BC_7F_2) individuals for inheritance analysis. The F_1 plants of this population were not confirmed to be heterozygous until they were evaluated for resistance to BYMV-NY.

Genomic DNA for restriction enzyme evaluation, adapter-titration experiments, and GBS library construction was obtained by harvesting ~1cm^2 (~50 mg) of the tip of a young expanding trifoliate leaf (V1), grinding to a fine powder under liquid nitrogen, and then using the Qiagen Plant DNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. The total final elution volume was 40 µL. The DNA was confirmed to be of high molecular weight, and of high purity and quantity by 1.5% agarose gel electrophoresis with a 2.5 kb Molecular Ruler (Bio-Rad Laboratories) and restriction digestion at 37°C for 1 h with *Hind*III (New England Biolabs). DNA was also checked for purity and quantity on a NanoDrop ND -1000 spectrophotometer (Thermo Fisher Scientific), and dsDNA was quantified with the Quant-iT PicoGreen dsDNA quantification kit (Life Technologies) and a Synergy 2 multi-mode microplate reader (Biotek Instruments). The DNA was diluted to 10 ng/µL and arrayed on a plate in preparation for genotyping. Genomic DNA from the parents,

F_1, and F_2 plants of the Hystyle x B28S2C population used for cosegregation analysis was obtained by a 96-well plate adaptation of a standard DNA extraction protocol for common bean (Afanador et al., 1993).

4.2.2 Virus isolates and interaction phenotypes

The 'New York' isolate of BYMV (BYMV-NY) was recovered from a symptomatic plant in a snap bean production field in Avon, NY in August of 2007. This isolate was confirmed to be a pure BYMV isolate by host range, symptomatology, serology, and RT-PCR. The 'New York' isolate of ClYVV (ClYVV-NY) was acquired from the Rosario Provvidenti collection at the New York State Agricultural Experiment Station (NYSAES) (Provvidenti and Schroeder, 1973) and its interaction with common bean has been characterized in detail by recent research (Larsen et al., 2008; Hart and Griffiths, 2013). A series of *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), BYMV, and ClYVV host differential genotypes possessing various dominant and recessive potyvirus resistance alleles were infected and their interactions with BYMV-NY and ClYVV-NY were evaluated for further characterization of the isolates (Appendix 4.1). The isolates were maintained in frozen (-80°C) and desiccated leaf tissue of Hystyle and were multiplied in-vivo by periodical mechanical transfer to newly expanded primary leaves (VC) of Hystyle.

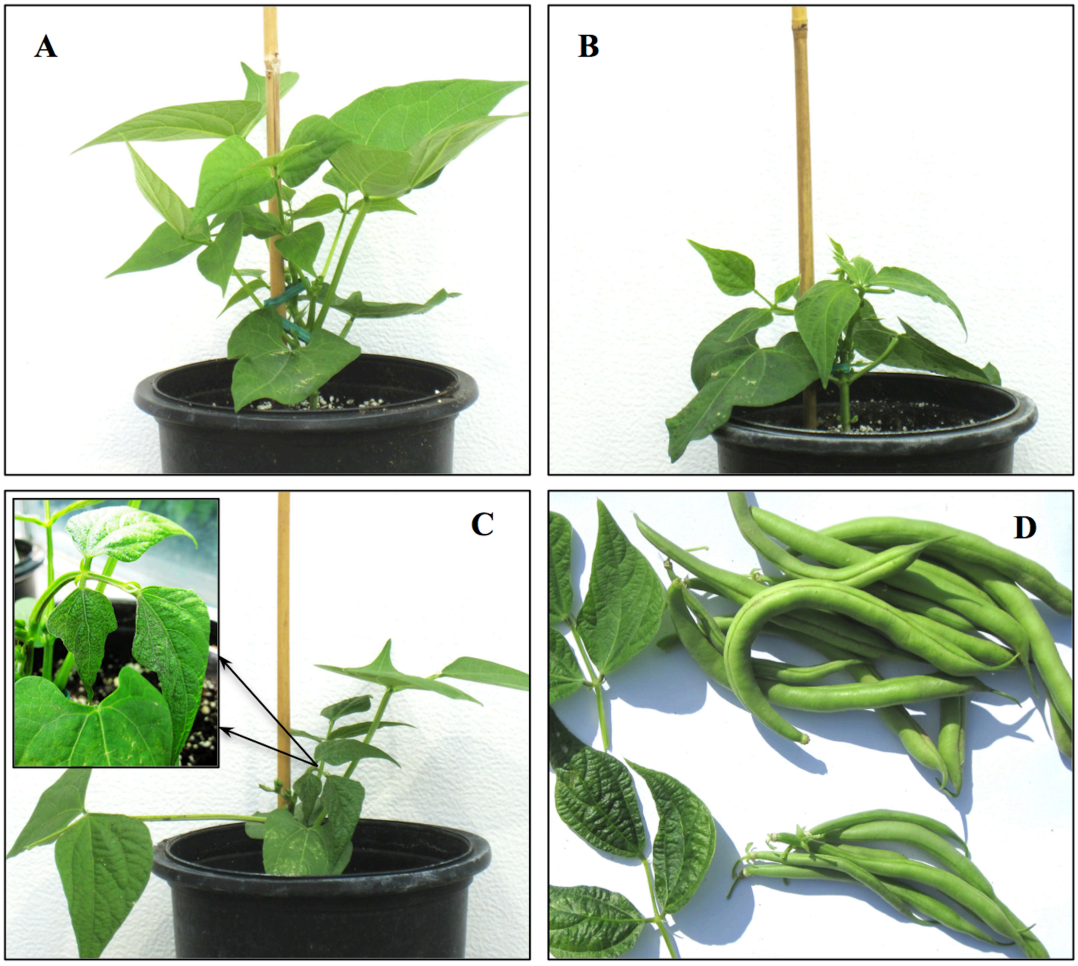
All plants were grown under research greenhouse conditions (temperature 24 day/21°C night; 14 h photoperiod) at the NYSAES where routine irrigation, fertilization, supplemental lighting, and integrated pest management regimes were

followed. The Seeds were planted in Cornell mix (Boodley and Sheldrake, 1972), in 10- by 10-cm cells of 18-cell flats (Speedling Inc., USA) Inoculum was prepared fresh by grinding partially expanded (~50%), symptomatic initial trifoliate (V1) leaves (1:10 w/v) in cold 0.01 M phosphate buffer (pH 7.0). All experimental material was dusted with 23 μm silicon carbide powder (Electron Microscopy Sciences) and inoculum was applied by gently rubbing the primary leaves (VC) with a triturate soaked pestle. Plants were rinsed with deionized water following inoculation. All appropriate experimental material was inoculated again 10 d post the first inoculation (dpi) to eliminate escapes from infection.

The interaction phenotypes of BYMV-NY and ClYVV-NY with the host differential genotypes, the 84 $BC_1F_{5:6}$ differential RILs, the four parental genotypes, and all of the material employed in the cosegregation analysis were defined by visual categorization of symptom expression. Symptom expression was categorized as resistant, susceptible, and in some cases, as resistant followed by delayed systemic necrosis and plant death. Visual resistance phenotypes for the 84 $BC_1F_{5:6}$ differential RILs were confirmed by progeny testing 9 plants each with BYMV-NY and ClYVV-NY. The visual resistance phenotypes for the parental, F_1, and F_2 populations of the BYMV-NY cosegregation analysis were confirmed by enzyme-linked immunosorbent assay (ELISA) of tissue samples from vegetative trifoliate (V2) leaves 15 dpi with the Potyvirus group monoclonal antibody according to the manufacturer's instructions (Agdia). Virus detection by RT-PCR (Hart and Griffiths, 2010) was also carried out on the parents. A resistance phenotype was defined by the complete absence of visual symptoms and negative ELISA results where absorbance values at OD_{405} were less

than two times that of the negative controls. Visual categorization of symptom expression was recorded initially at 10 dpi, plants were then potted up into 16.5cm- by 16.5-cm pots, and then evaluated again at 22, 30, 45, 60, and 90 dpi. Visual phenotypes were acquired with reference to positive and negative controls in all cases where homozygous genotypes were available. Examples of BYMV-NY interaction phenotypes are illustrated in Figure 4.1.

Figure 4.1. Example of BYMV-NY interaction phenotypes in the parents and progeny of the Hystyle x B28S2C cosegregation populations. A) Resistance phenotype conditioned by *By-2* in the snap bean breeding line B28S2C. B) Susceptibility phenotype in the snap bean cultivar Hystyle. C) Delayed systemic necrosis phenotype in an initially symptomless F_2 individual that ultimately caused premature death. A close-up of the necrosis response is inset. D) Example of fresh pod yield from a healthy field-grown Hystyle plant on the top, and the fresh pod yield of a field-grown plant infected with BYMV-NY at the seedling stage on the bottom.



4.2.3 Genotyping-by-sequencing

The suitability of *Ape*KI and *Pst*I as restriction enzymes for complexity reduction and genotyping-by-sequencing (GBS) in *P. vulgaris* were evaluated with previously published protocols, adapters, barcodes, and primers (Elshire et al., 2011; Appendix 4.2). The adapters were obtained from the Institute for Genomic Diversity at Cornell University. Briefly, a series of eight test libraries for each enzyme were constructed in parallel by restriction digestion of 200 ng of Hystyle DNA with *Ape*KI (75°C for 2 h) and 500 ng of Hystyle DNA with *Pst*I (37°C for 2 h) in 20 µL volumes containing 2 µL of 10x buffer and a tenfold excess of restriction enzyme (New England Biolabs). Enzyme-specific adapters were added to each of the eight test libraries of restriction fragments in eight different quantities (1.8, 2.4, 3.6, 4.2, 4.8, 5.4, 6.0, and 7.2 ng) to construct an adapter titration. The adapter titration allowed for an empirical determination of the correct ratio of adapters to restriction fragment sticky ends for the enzyme and genome of interest (Elshire et al., 2011). The adapters were ligated to the restriction fragments in 50 µL volumes containing 20 µL of digested genomic DNA from the previous step, 5 µL of 10x ligase buffer, and 1 µL of T4 ligase (400 CELU/ µL) (New England Biolabs) for 60 min at 22°C, and then for 30 min at 65°C for ligase denaturation. These adapter-ligated fragments were then purified with a QIAquick PCR cleanup kit according to the manufacturer's instructions (Qiagen).

The test libraries of adapter-ligated fragments were PCR-amplified to complete the library construction. PCR was performed in a 50 µL volume that consisted of 10 µL of purified adapter-ligated fragments, 25 µL of *Taq* 2x Mastermix (New England

Biolabs) and 12.5 pmol of each PCR primer (Supplemental Table S2). The PCR protocol consisted of one 5 min cycle at 72°C, followed by one cycle at 98°C for 30 s, followed by 18 cycles of 98°C for 10 s, 65°C for 30 s, 72°C for 30 s, and one final 5 min cycle at 72°C and was performed on a Gradient Master Cycler PCR System (Eppendorf). The PCR amplified test libraries were then purified with a QIAquick PCR cleanup kit. The purified PCR-amplified libraries were evaluated with an Agilent 2100 Bioanalyzer (Agilent Technologies) and the resultant electropherograms were examined for the presence, concentration, and fragment sizes of the sequencing library, the presence of library peaks (i.e. repetitive DNA sequences), and for the occurrence of adapter dimer in each of the 8 libraries of the adapter titration.

The results of the test libraries and adapter titrations were considered in the broader context of the additional variables and goals of the project, and this informed our choice of enzyme as *Ape*KI and an optimal adapter concentration of 1.5 ng of adapter to 50 ng of *P. vulgaris* DNA. We constructed a 96-plex *Ape*KI GBS library composed of the 84 differential RILs and three replicate samples from each of the four parental genotypes. DNA of the 96 individuals was normalized to 10 ng/μL and arrayed on a plate according to a key file that was created to define the position and barcodes for each individual sample in the library (Appendix 4.3). The GBS library construction protocol for the germplasm was similar, except that 5 μL (50 ng) of genomic DNA from each individual was digested with *Ape*KI and 1.5ng of *Ape*KI barcoded-adapters were ligated to the subsequent restriction fragments as previously described except that the reactions were carried out in a 96 well plate. The adapter-ligated fragments from each well (2 μL each) were then pooled together into a 1.5 ml

tube containing 1000 µL of QIAquick PCR cleanup kit binding buffer. The pooled fragments were purified with the QIAquick PCR cleanup kit and eluted into a final volume of 50 µL. The multiplexed library was then PCR-amplified as previously described except that 8 µL of the library was used as the template in the reaction. After final purification of the PCR product, the GBS library was eluted in a final volume of 30 µL and analyzed by the Agilent 2100 Bioanalyzer, quantified, and diluted to 3.6 pM for sequencing according to the GBS protocol (Elshire et al., 2011). The GBS library was submitted to the Weill Cornell Medical College Genomics Resources Core Facility for 101-cycle single end only sequencing on one lane of a 16-lane flow cell of an Illumina HiSeq 2000 instrument (Illumina).

4.2.4 Sequence alignment and SNP calling

The sequencing reads were processed with the GBS Discovery Pipeline for species with a reference genome implemented in TASSEL Version 3.0 (Bradbury et al., 2007) and following the pipeline documentation (Glaubitz et al., 2013). In conjunction with the key file, the pipeline identifies high quality unique sequence reads, termed tags, that contain a barcode, a cut site, and accurate genomic sequence, and then merges these tags, indexes them, and then aligns them to the reference genome. The sequence tags for our GBS library were aligned to the v1.0 release of the *P. vulgaris* genome (DOE-JGI and USDA-NIFA, 2013) using the Burrows-Wheeler Alignment (BWA) (Li and Durbin, 2009) command of the pipeline. The Discovery SNP Caller was implemented to align the multiple sequence tags from the same physical locations across the genome, to call SNPs at these locations across the

individual samples, and then to output this data into one HapMap format file (.hmp.txt) per chromosome for downstream analysis. The exact commands and arguments used with the pipeline to process the dataset described here are outlined in Appendix 4.4. Missing SNP data was imputed with a novel algorithm currently in development at the Institute for Genomic Diversity (K. Swarts, personal communication, 2013).

4.2.5 Genome-wide association study

To discover associations between the genome-wide SNPs and the virus interaction phenotypes in our germplasm we conducted a case-control genome-wide association study (GWAS) using a compressed mixed linear model (Zhang et al., 2010) implemented in the Genome Association and Prediction Integrated Tool (GAPIT) R package (Lipka et al., 2012) in R v.3.0.2 (R Core Team, 2013). Due to the significant population structure that exists in *P. vulgaris* (Mamidi et al., 2011) we chose to omit the black bean NILs from our snap bean-centered study in our initial conservative analysis of the data. The analysis employed the SNP dataset from the 84 differential RILs and the snap bean parents Hystyle and Tapia, and was composed of 7,530 SNPs, each with minor allele frequency (MAF) greater than or equal to 0.05. With this snap bean only dataset, we implemented GAPIT to automatically calculate the kinship matrix (Van Raden, 2008), and to perform forward model selection to determine that zero was the optimal number of principal components to control for population structure. GWAS was performed with the default clustering algorithm (average) and group kinship type (mean) of GAPIT. A SNP was considered to be

significantly associated with the *By-2* resistance phenotype if the *P*-value was less than the Bonferroni adjusted alpha of 0.01 or $P < 1.3 \times 10^{-6}$.

4.2.6 Cosegregation analysis

We identified a subset of SNPs that were discovered and genotyped by GBS and that were contained within the physical interval delimited by the GWAS results. Seven SNPs within this interval were selected as candidates for conversion to Kompetitive Allele Specific PCR (KASP) assays (LGC Genomics) and for subsequent use in inheritance and cosegregation analyses. The physical positions of the SNPs were located on the reference genome, and at least 50 bp of genome sequence flanking each side of each SNP was submitted to the KASP by Design (KBD) service (LGC Genomics) for KASP assay primer synthesis. The primer sequences are listed in Appendix 4.5. DNA was isolated from the cosegregation population and parents, and KASP assays were performed in 8 µL reaction volumes containing 20 ng of template DNA, 4 µL of 2x KASP Reaction Mix, and 0.11 µL of the primer assay mix. PCR amplification was completed with one 15 min cycle at 94°C, followed by 15 cycles of 94°C for 20 s, 65-57 °C for 60 s (dropping by 0.8°C per cycle) and 57°C for 60 s using the Gradient Master Cycler PCR instrument. KASP genotype calls were obtained with the ViiA7 Real-Time PCR System (Life Technologies) by implementing the 'genotyping experiment' module of the instrument's software. To acquire the most complete SNP cosegregation dataset, allele calls were checked manually and some were re-scored from unassigned to an allele call based on the interpretation of their fluorescence data and the clustering with other called SNPs (see Semagn et al., 2013).

4.3 RESULTS

4.3.1 *By-2* resistance to BYMV-NY and ClYVV-NY

Throughout the course of the *By-2* introgression into snap bean and the development of the RIL populations we observed a third interaction phenotype where resistant individuals (Figure 4.1A) developed a localized necrosis in secondary veins of trifoliate leaves and subsequently succumbed to systemic necrosis. This delayed systemic necrosis response (Figure 4.1C.) occurred in every phenotyping experiment, but was more frequent under lower natural light and lower temperature conditions in the greenhouse in winter. The delayed systemic necrosis also occurred in the progeny of resistant plants selected in previous generations and there was no apparent or consistent segregation ratio for the number of plants with delayed systemic necrosis in the progeny of the resistant RILs (Table 4.1). Necrotic tissue, and non-necrotic tissue on necrotic plants remained ELISA negative (data not shown). This led us to hypothesize that the cause of the delayed systemic necrosis response may be due to reduced penetrance of resistance *By-2* conditioned by epistatic interactions, genetic background, and/or low-temperature sensitivity.

The inheritance of resistance and the frequency of delayed systemic necrosis in two additional F_2 populations, Hystyle x B28S2C, and BT-1 x B-21, consisting of 200 and 167 F_2 individuals respectively, both evaluated under low temperature conditions, revealed additional evidence for low-temperature sensitivity of resistance (Appendix 4.6). In both F_2 populations, the segregation ratio of resistant to susceptible plants conformed to a Mendelian segregation ratio (3:1) for a major dominant gene, but 88 out of 159 resistant plants in the Hystyle x B28S2C population, and 99 out of 121

resistant plants in the BT-1 x B-21 population exhibited delayed systemic necrosis. The frequency of delayed systemic necrosis suggests that resistance is incompletely dominant under these experimental conditions, but the ratios of necrotic plants do not conform to the model for an incompletely dominant allele (Appendix 4.6). The ratios of interaction phenotypes were acquired in a third F_2 population, the Hystyle x B28S2C F_2 population that was employed for SNP-cosegregation analysis and was phenotyped in the greenhouse in summer. The phenotypic ratios from that population are presented in the cosegregation analysis section below.

4.3.2 Genotyping-by-sequencing in common bean

The enzyme evaluation electropherograms provided insight into the size and nature of the adapter-ligated restriction fragments produced by the digestion of the *P. vulgaris* genome with either *Ape*KI or *Pst*I. Example electropherograms are provided for each enzyme in Appendix 4.7. There were numerous irregular peaks in the *Ape*KI library and these results suggested that *Ape*KI cut sites occur frequently in repetitive regions of the *P. vulgaris* genome. We examined this possibility by adding one barcoded *P. vulgaris* sample to an *Ape*KI GBS library for another project as a test run. The sequences associated with this sample contained approximately 15% overrepresented sequence with high similarity to the *P. vulgaris* chloroplast genome (data not shown), and this could be a possible explanation for the irregular peaks in the library. In contrast, *Pst*I produced a library of adapter-ligated fragments with a smooth curve and without irregular peaks, and also tended to produce less concentrated libraries with smaller fragments (Appendix 4.7). Despite the more regular appearance

of the *Pst*I library, we chose to employ *Ape*KI to construct the GBS library for this research because its 5 bp recognition sequence (GCWGC) was presumed to occur at a higher frequency than that of the 6 bp recognition sequence of *Pst*I (CTGCAG), and we wanted to maximize the number of SNPs detected in our highly related germplasm.

The adapter titration experiments provided clear results in that throughout the range of the eight adapter concentrations, adapter-dimer was not appreciable for either enzyme. Therefore we chose an *Ape*KI adapter quantity (1.5 ng adapter to 50 ng genomic DNA) that routinely produced high quality libraries. The sequencing resulted in 164,308,166 -101 bp reads, approximately 16.6 Gb of sequence data. The mean phred score was 34.23 across all bases, and 0% of the reads contained uncalled bases (N's) demonstrating the high quality of the data. The GBS discovery pipeline was implemented to filter the raw sequencing reads and accept only those reads that were of good quality (no adapter dimer, no N's in the first 72 bp) and that contained identity with one of the barcodes in the key file and the *Ape*KI cut site. This resulted in a total of 131,349,075 quality reads for our library, with a mean number of reads per individual of 1,368,219, a standard deviation (SD) of 739,179, and a coefficient of variation (CV) of 54%. Three plants of each of the four parental genotypes were sampled and sequenced as technical replicates as well as to achieve deeper coverage. The read numbers for B-21, BT-1, Hystyle, and Tapia were 2,676,921 (mean=892,307; SD = 202,766; CV=22%), 3,529,040 (mean=1,176,346; SD=386,776; CV=32%). 776,398 (mean = 258,799; SD = 160,705; CV = 62%) and 3,344,042 (mean = 1,114,680; SD= 96,754; CV= 9%) respectively. The number of reads per sample across all of the samples is displayed in Appendix 4.8. Three samples, F5-12-

7, F5-27-6, and F5-39-3 had very low read numbers likely due to reduced quality DNA.

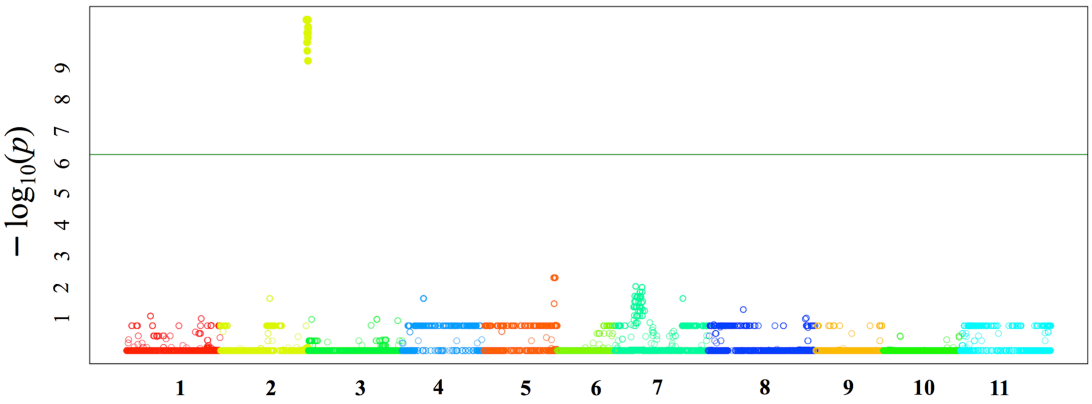
Of the total 131,349,075 quality sequencing reads, 122,695,153 or 93.4% were aligned to physical positions by BWA on the *P. vulgaris* v1.0 reference genome (DOE-JGI and USDA-NIFA, 2013). The GBS Discovery SNP Caller was then implemented to call SNPs and to filter the results. In total, 374,603 unique sequence tags resulted in the identification of 19,575 SNPs across the 11 assembled pseudomolecules and 69 SNPs from 39 unmapped scaffolds. We filtered and retained SNPs with a minimum inbreeding coefficient of 0.9, and SNP call rates of at least 10% across all samples. With these filtering parameters we obtained a total of 18,407 SNPs, with a range of 17-40% missing data per chromosome, and an overall mean of 29% missing data across the genome. This dataset allowed us to retain one SNP every 30.3 kb on average across the entire genome when considering all of the germplasm. The number of SNPs per pseudomolecule was moderately correlated with the length of the respective pseudomolecule (correlation coefficient = 0.45) (Appendix 4.9), although our germplasm is not an ideal dataset to examine this relationship given its small sample size, narrow genetic base, and history of strong selection.

4.3.3 Genome-wide association study for *By-2* virus resistance

A case-control GWAS was conducted to identify SNPs associated with resistance to BYMV and ClYVV by considering the 42 susceptible RILs, Hystyle, and Tapia as the cases, the 42 resistant RILs as the controls (Table 4.1), and by surveying the genetic variation across these lines with 7,530 SNPs (MAF $\geq$ 0.05) that segregated

within this germplasm. A compressed mixed linear model was implemented to control for familial relatedness (Appendix 4.10), and a conservative Bonferroni adjusted alpha of 0.01 ($P \leq 1.3 \times 10^{-6}$) was set as the threshold for significant association. This analysis resulted in the identification of 44 SNPs strongly associated with *By-2* potyvirus resistance, and that delimited a 974 kb physical region (Chr02: 47991715 – 48965798) on the distal portion of chromosome 2 (Figure 4.2). The results of the GWAS are also presented on a chromosome-by-chromosome format in Appendix 4.11 and in a table in Appendix 4.12. We conducted a similar association analysis with 15,585 SNPs (MAF $\geq$ 0.05) that included the black bean NILs, (BT-1 and B-21), and an additional 8,055 SNPs that were private to these lines to attempt to uncover SNPs in closer proximity or in stronger association with *By-2*. A Bonferroni adjusted alpha of 0.01 imposed a *P*-value of 6.4×10^{-7} as the threshold for significance for this analysis. The results were similar to the snap bean-only dataset except that a total of 130 SNPs were identified within a 66.7 kb larger interval in the same physical location (Chr02: 47989185 – 49030001). Because the results did not differ significantly outside of additional SNP discovery in the black bean NILs, those results are not presented here.

Figure 4.2 Case-Control genome-wide association study (GWAS) for *By-2* potyvirus resistance in a population of 44 susceptible and 42 resistant recombinant inbred lines (RILs) from a snap bean breeding program. The association of 7,530 single nucleotide polymorphisms (SNPs) (represented by open circles) is plotted as $-\log_{10}$ transformed *P* values on the y-axis against the physical positions of the 11 chromosomes of the common bean genome on the x-axis. The green horizontal line indicates the $-\log_{10} P$ value threshold of the least statistically significant SNP as predicted by Bonferroni adjustment ($P \leq 1.3 \times 10^{-6}$). The GWAS identified 44 SNPs associated with *By-2* virus resistance that delimit a 974 kb region on the distal portion of chromosome 2.



We also attempted to explore if there was a genetic basis for the delayed systemic necrosis response in initially symptomless individuals by conducting GWAS on this trait in the snap bean only dataset based on the phenotypes of the progeny testing (Table 4.1). Cases were considered on a line basis, where if any of the $BC_1F_{5:6}$ individuals out of the ~9 plants tested exhibited delayed systemic necrosis, the BC_1F_5 individual that was genotyped was considered as a case. No significant associations were detected with any of the SNPs, even when we imposed a less conservative threshold of a genome-wide false discovery rate (FDR) of 10% (Benjamini and Hochberg 1995). The results of this analysis are presented in Appendix 4.13, although this analysis was underpowered to detect smaller effect alleles that may be involved in conditioning the delayed systemic necrosis response due to the small sample size.

Additional research is needed to definitively rule out the role of genotype, genotype by genotype, or genotype by environment interaction as the basis for delayed systemic necrosis.

4.3.4 Cosegregation analysis

A series of single-marker KASP assays were developed to confirm and validate the cosegregation of SNPs located in the distal portion of chromosome 2 that were presumed to be in linkage disequilibrium with virus resistance conditioned by *By-2*. We chose seven candidate SNPs to convert to single marker assays within the 974 kb region (Table 4.2; Appendix 4.5) identified by the GWAS. Ten individual plants of each of the parents B28S2C and Hystyle, and 18 of their F_1 and 185 of their F_2 progeny were phenotyped for resistance to BYMV-NY by multiple inoculations, visual assessment, and ELISA. We progeny tested nine plants from each of five F_2 individuals that were phenotyped as susceptible but that did not achieve positive ELISA values and confirmed their susceptibility to BYMV-NY. DNA was isolated from the individual plants of the parents and pooled together by parent, and from all F_1 and F_2 plants to be used as template for the KASP assays.

The results presented in Table 4.2 and Appendix 4.6 provide strong evidence that *By-2* is a single dominant allele that may deviate from complete dominance to condition resistance to BYMV-NY. The genotypic segregation ratios of all of the KASP assays fit the expected Mendelian ratio for a single codominant marker and indicated that the F_2 population was not exhibiting segregation distortion in the chromosomal region presumed to harbor *By-2* ($P = 0.84$) (Table 4.2). If the phenotype

is considered as two distinct classes, that is, individuals that exhibited the pronounced stunting and mosaic symptoms of susceptibility, and those that did not and were considered resistant, the ratio of 141 resistant individuals to 44 susceptible conforms to the Mendelian ratio expected for a single dominant gene ($P = 0.70$) (Table 4.2). In all seven of the KASP assays tested, the 45 F_2 individuals that were typed as homozygous for the dominant B28S2C resistance (R) allele remained resistant throughout the course of the entire experiment as verified by ELISA. Seventy-eight additional F_2 individuals that were typed as heterozygous (H) for all seven KASP assays were also verified visually and by ELISA as resistant throughout the course of the experiment. Eighteen F_2 individuals typed as H for all of the KASP assays were

Table 4.2. Cosegregation of KASP SNP alleles with resistance or susceptibility to BYMV-NY as conditioned by the dominant *By-2* allele in the Hystyle x B28S2C populations. The shaded region of the table corresponds with a deviation from SNP allele-*By-2* resistance cosegregation where a putative recombinant individual was identified.

Pop. †	Phenotype‡	No.§	SNP Allele¶	Pv02_ 48722161#	Pv02_ 48790627	Pv02_ 48843877	Pv02_ 48849943	Pv02_ 48874335	Pv02_ 48891077	Pv02_ 49012008
Hystyle	S	10	S	10	10	10	10	10	10	10
B28S2C	R	10	R	10	10	10	10	10	10	10
F_1	R	17	H	17	17	17	17	17	17	17
	R(dSN)	1	H	1	1	1	1	1	1	1
F_2 ††	R	45	R‡‡	45	45	45	45	45	45	45
		78	H	78	78	78	79	79	79	79
	R(dSN)	18	H	18	18	18	18	18	18	18
	S	44	S	44	44	44	43	43	43	43

† Pop. = Populations utilized in the cosegregation analysis.
‡ Response to infection with BYMV/ClYVV, R = resistant – no symptoms; S = susceptible – pronounced stunting and mosaic; R(dSN) = Initially classified as resistant (R), then exhibited delayed systemic necrosis = (dSN). The phenotypes assigned were based on multiple inoculations, visual assessment, ELISA, and in some cases by progeny testing.
§ No. = The number of individual plants with a given phenotype
¶ KASP assay SNP allele, R = B28S2C allele; S = Hystyle allele; H = heterozygous.
# KASP assay ID, Pv02_ = chromosome 02 followed by the physical location of the assayed SNP in bp. DNA from the 10 plants of each parent was pooled prior to genotyping.
†† Expected phenotypic ratio for F_2 population of 3R [(including R(dSN)]:1S; observed 141-R:44-S; $\chi^2 = 0.145$, $P = 0.70$ ($df = 1$).
‡‡ Expected genotypic ratio for F_2 population of 1-RR:2-H:1-SS; observed e.g. Pv02_48722161, 45RR:96H:44S; $\chi^2 = 0.350$, $P = 0.84$ ($df = 2$).

initially categorized as resistant, verified by ELISA, and subsequently exhibited delayed systemic necrosis at varying developmental stages over the course of the phenotyping experiment. These 18 individuals provide evidence for the deviation from complete dominance. In addition, one F_1 individual out of the 18 typed as H as expected also exhibited the delayed systemic necrosis phenotype and further suggested deviation from complete dominance. The 44 F_2 individuals that exhibited pronounced stunting and mosaic symptoms of susceptibility were typed accurately as homozygous for the Hystyle susceptibility (S) allele at all seven of the KASP assays except for one F_2 individual that was typed as S for assays Pv02_48722161 through Pv02_48843877 and typed as H for assays Pv02_48849943 through Pv02_49012008 (see shaded regions of Table 4.2). This individual was presumed to have a recombination event between Pv02_48843877 and Pv02_48849943 suggesting that the physical position of the *By-2* candidate gene is upstream of Pv02_48849943. Though we are tentative about this specific conclusion because it is based on one individual in a modestly sized mapping population. The results of our cosegregation analysis provide strong evidence that the SNPs that were discovered and genotyped within the 974 kb physical interval are in linkage disequilibrium with *By-2* virus resistance on the distal portion of chromosome 2.

4.4 DISCUSSION

GBS was successfully adapted and applied to common bean in concert with the recently released reference genome (DOE-JGI and USDA-NIFA, 2013) to

simultaneously discover and genotype a total of 19,575 SNPs in our germplasm. SNPs were discovered across all 11 chromosomes of common bean and appear to be relatively evenly distributed along each chromosome (Appendix 4.9 and 4.11). This is an unprecedented number of SNPs to have available for a genetics study common bean, and it is anticipated that lower coverage sequencing of more diverse populations and or diversity panels, combined with imputation, could result in the discovery of many tens of thousands of additional SNPs for common bean genetics. Indeed, the common bean genome seems particularly well-suited to reduced representation sequencing due to its small size (521 Mb [DOE-JGI and USDA-NIFA, 2013]), and the relatively low levels of duplication and repetitive sequences in comparison to other plants (Gepts et al., 2008). The investigation of additional restriction enzymes, alternate library construction techniques (Poland et al., 2012; Sonah et al., 2013), and novel alignment and imputation algorithms (Spindel et al., 2013) presents a rich set of tools that are emerging to further adapt and customize GBS for allele discovery in common bean.

The multiplexed library construction was technically simple and the entire process from DNA extraction to sequencing was rapid. There was considerable variation in read number per sample that was likely due to variation introduced by DNA quality differences, DNA quantification, and manual pipetting errors. Overall the amount of missing data was relatively low because of the high level of sequencing coverage achieved and the redundancy that we included in our library construction by including the DNA of seven individuals from each of the 12 $BC_1F_{4:5}$ differential RILs. Subsequent filtering of the dataset allowed for a total of 15,585 SNPs with MAF ≥

0.05 to be retained for genotype-phenotype analysis.

The initial approach to establishing the genotype-phenotype relationship was somewhat similar to a bulked segregant analysis (BSA) for a major disease resistance allele where a small number of individuals that are resistant and a small number of individuals that are susceptible are selected and the DNA is pooled according to the phenotype to identify the DNA polymorphisms that are shared by each individual in the pool and therefore correspond to the resistance region (Michelmore et al., 1991). The advantage of the barcoded multiplexed library construction of GBS is that individual samples do not need to be pooled together. This allows the genotype of each individual to be observed, and the segregation of all genotyped SNPs can be confirmed to reduce and eliminate problems associated with incorrectly phenotyped individuals.

Our approach differs significantly from BSA in how the genotype-phenotype relationships are established. By genotyping lines and individuals that shared parents and originated from the same series of crosses that would be typical of any self-pollinated crop improvement program, a population of closely related individuals was available for analysis. This population removed the effect of cryptic population structure in the promotion of false positives, and allowed for kinship matrix to be used to control for familial relatedness so that a case-control GWAS approach and a statistical model could be used to test the significance of genotype-phenotype associations. To the best of our knowledge, this novel approach for identifying markers in linkage disequilibrium with major genes has not been demonstrated previously.

This GWAS based approach identified 44 highly significant SNPs in the snap bean germplasm that delimited a 947 kb region on the distal portion of chromosome 2. This physical position spans the region between Phvul.002 47991415 to Phvul.002 48965798. The incompletely dominant, high-temperature sensitive *I* allele that conditions a range of resistance and necrosis responses to BCMV and BCMNV (Collmer et al. 2000) and provides a broad spectrum of resistance to a number of other related potyviruses (Fisher and Kyle, 1994) has been mapped to this general region (Freyre et al., 1998; Vallejos et al., 2000). Genbank (Benson et al., 2013) queries of the cloned DNA sequences associated with the *I* locus in the in-depth molecular characterization of this region (DQ002468-DQ002476) (Vallejos et al., 2006) and BLASTN analysis to the common bean genome with Phytozome (Goodstein et al., 2012) revealed that the physical region for this allele is positioned within an 81.7 kb region from Phvul.002 48183168 to Phvul.002 48264877, and that this region is positioned within the larger 947 kb region identified by the GWAS for *By-2* here.

The *I* locus was demonstrated to harbor a complex cluster of Toll/interleukin-1 receptor-nucleotide binding site-leucine rich repeat (TIR-NBS-LRR)-type virus resistance features prior to the release of the reference genome, but the reference confirmed that there are seven loci with several alternate transcripts in the region predicted to have similar homology. These TIR-NBS-LRR types of features have previously been confirmed as the molecular basis for the major dominant virus resistance allele *N* in *Nicotiana* spp. to *Tobacco mosaic virus* (TMV) (Whitham et al., 1994) and *Y-1* in *Solanum tuberosum* to *Potato virus Y* (PVY) (Vidal et al., 2002). Both of these TIR-NBS-LRR alleles condition temperature sensitive hypersensitive

necrosis in response to virus infection.

The *By-2* allele exhibits many similarities to the *I* allele in that it also appears to be temperature sensitive, incompletely dominant, and in that it conditions a spectrum of resistance and necrosis that includes more than one virus species. Although there are numerous additional candidate genes in the 974 kb region, it is possible that *By-2* is a unique resistance allele at the *I* locus. Unfortunately the genetic recombination that we sampled in the region could not resolve this hypothesis, and it is important to note that recombination was reported to be considerably suppressed around the *I* locus in previous research (Vallejos et al., 2006). Additional recombination in the *By-2* / *I* region is needed and will be the subject of future effort. Apart from the fact that the resistance spectrum of *By-2* includes BYMV and ClYVV, one particularly striking difference between these two factors is that *By-2* appears to be sensitive to low temperatures, and *I* appears to be sensitive to high-temperatures (Collmer et al., 2000). Additional research is needed to confirm the effects of temperature, genetic background, and allele dosage on the expression of this resistance.

While this research has identified many hypotheses as the subject for future research, it has fulfilled all of the objectives set forth and made a valuable contribution to the study and understanding of virus resistance in common bean as well as the application of genomics to common bean improvement. We employed a GBS protocol, publicly available bioinformatics tools, and developed a novel strategy to identify and validate highly significant SNPs that were associated with the *By-2* potyvirus resistance allele in our germplasm to a relatively narrow region on

chromosome 2 where known virus resistance features are present. This research generates new knowledge and hypotheses in common bean-potyvirus interactions, develops important tools for future research and fine-mapping, and enables new opportunities for marker-assisted gene pyramiding. These advances should assist directly in the development of multiple-virus resistant snap bean cultivars for the Great Lakes Region of the United States and beyond.

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CHAPTER 5

ENABLING THE DEVELOPMENT OF MULTIPLE-VIRUS-RESISTANT SNAP BEANS: CONCLUSIONS AND FUTURE RESEARCH

This research applied recent advances in the knowledge of host plant resistance to viruses and in plant molecular biology to further identify, characterize, and evaluate the genetics of natural resistance to two economically important potyviruses of common bean (*Phaseolus vulgaris* L.), *Bean yellow mosaic virus* (BYMV), and *Clover yellow vein virus* (ClYVV). The assembly and examination of an informative panel of 21 common bean genotypes with previously characterized resistance to ClYVV allowed for the three previously identified resistance alleles *cyv*, *desc,* and *bc-3,* to be resolved into an allelic series at the *Bc-3* potyvirus resistance locus as established by allelism testing. The gene symbol nomenclature of the putatively independent alleles *cyv* and *desc* was revised to *bc-3*^{2} to reflect the order in which the alleles were discovered, as well the differential susceptibility to strain NL 3 D of *Bean common mosaic necrosis virus* (BCMNV) that it conditions in contrast to the *bc-3* allele.

In-depth analysis of a candidate gene, *P. vulgaris* eukaryotic translation initiation factor 4E (*PveIF4E*), was undertaken with the hypothesis that subtle mutations in the coding sequence conferred effective resistance to ClYVV, and ClYVV and NL 3 D in *bc-3*^{2} and *bc-3* genotypes, respectively. The analysis revealed non-synonymous single nucleotide polymorphisms (SNP) that were able to predict

resistance or susceptibility across the 21 informative genotypes and in all of the F_2 individuals of three separate segregating populations when genotyped by allele-specific molecular markers. This analysis established two key non-synonymous SNPs, C227A as the putative functional determinant for ClYVV conditioned by *bc-3*^{2} and A332G as the putative functional determinant for BCMV/BCMNV resistance conditioned by *bc-3*. The *PveIF4E* allele-specific molecular markers that were designed are rapid and user-friendly for high-throughput marker-assisted selection of *bc-3* resistance alleles in bean improvement programs.

To further investigate the relationship of non-synonymous SNPs in *PveIF4E* with resistance to ClYVV, a large and representative panel of common bean genetic diversity was assembled and included 391 accessions of the USDA-ARS core collection, 99 snap bean cultivars and breeding lines, and 63 dry bean cultivars and breeding lines with known differential responses to ClYVV and/or BCMV/BCMNV. This panel was evaluated for resistance to ClYVV, and resistant accessions were genotyped with the *PveIF4E* allele-specific molecular markers. The results of the phenotyping allowed for the identification of ClYVV resistance, mostly conferred by *bc-3*^{2}*,* in all major market classes of common bean. All of the resistant accessions were demonstrated to possess the C227A non-synonymous SNP. This further validated its key role in conferring resistance to ClYVV and demonstrated the utility of the allele-specific molecular markers for bean improvement and allele mining in germplasm collections. The phenotypic screen of the panel also led to the identification of ClYVV resistance in bean genotypes that possessed the *bc-u*, *bc-2*^{2} allele combination for resistance to BCMV/BCMNV and in bean genotypes that

possessed the *By-2* allele for resistance to *Bean yellow mosaic virus* (BYMV). These results provide the initial evidence to establish a revised model for ClYVV resistance in common bean that includes resistance alleles at independent loci (*bc-u bc-2*^{2}, *bc-3, By-2*) and provides a novel opportunity for resistance allele pyramiding to potentially enhance the durability of resistance to ClYVV in common bean.

To elucidate the molecular genetics of resistance to BYMV and ClYVV as conditioned by *By*-2, an allele that was originally introgressed from *P. coccineus* and that had never been mapped nor characterized at the molecular level, recently emerged genomics resources and genotyping techniques for common bean were adapted and employed. The recent completion of a reference genome sequence for common bean (*P. vulgaris* v1.0; DOE-JGI and USDA-NIFA, 2013) provided an unprecedented tool for allele discovery, but efficient methods for identifying and surveying DNA polymorphism throughout the genome were still lacking for establishing saturated linkage maps of genome-wide molecular markers. In order to overcome this constraint, the novel approach known as genotyping-by-sequencing (GBS) was adapted for common bean to leverage the powerful advances in data output and cost effectiveness of next-generation sequencing technologies. GBS was employed to discover and genotype 19,575 genome-wide SNPs that segregated in a unique set of *By-2* resistant and *by-2* susceptible recombinant inbred lines (RILs) that resulted from a snap bean *By-2* introgression program.

These SNPs were tested for their association with the resistance phenotype by conducting a case-control genome-wide association analysis (GWAS). Forty-four SNPs were found to be highly significant for association with virus resistance

conditioned by *By-2* and delimited a 947 kb physical region of the distal portion of chromosome 2. Seven of the 44 highly significant SNPs were selected for the design of allele-specific molecular markers to confirm that they cosegregated with *By-2* virus resistance in a separate segregating population. These markers were tightly linked with *By-2* and the results confirmed the physical location of *By-2* and validated the use of these allele-specific markers for marker-assisted selection in the breeding program. This research demonstrated the unprecedented abilities of GBS coupled with the common bean reference genome to discover the genetic variation that underlies economically important phenotypes in common bean.

Taken together, the results of the research have generated new knowledge and hypotheses, adapted new tools and techniques, and have enabled new opportunities to address the threat of aphid-transmitted viruses in snap bean production. These tools and techniques should be applied to the hypotheses that were unable to be addressed within the scope of this research. Both the research presented here, and suggested for the future can and should be applied directly to the breeding effort to develop multiple-virus-resistant snap beans.

PveIF4E **conditioned resistance to ClYVV and BCMV/BCMNV**

The research presented in Chapter 2 presented strong evidence that resistance to ClYVV and BCMV/BCMNV as conditioned by *bc-3* alleles is conferred by specific non-synonymous SNPs, but this was not confirmed by functional analysis. Given the precedent of this resistance mechanism to potyviruses in other plants, and the additional validation presented in Chapter 3, functional conferral is not necessary for

the deployment of this resistance. Given the simplicity of the mutations and the mechanism of resistance, it would nonetheless present a highly valuable proof-of-concept for the exploration of functional genomics tools such as virus-induced gene silencing vectors for common bean (Diaz-Camino et al., 2011).

Although not addressed here, the involvement of isoforms of eIF4E, and additional components of the translation initiation complex, such as eIF4G and eIF(iso)4G in recessive resistance to viruses in *P. vulgaris* should be further investigated. Previous research in common bean has ruled out involvement of eIF(iso)4E in recessive resistance to potyviruses (Naderpour et al., 2010), but not to geminiviruses, where recessive resistance alleles are also important (Blair and Morales, 2008). Cloning, sequencing, and alignment of eIF4G and its isoform have not been completed, and it may be possible that mutations in their coding sequences could play a role in resistance conferred by recessive resistance alleles at other loci in the bean genome (e.g. *Bc-u*, *Bc-1*, *Bc-2*). Previous research has demonstrated that eIF4E and eIF4G are involved in the multiplication of *Cucumber mosaic virus* (CMV) in the model plant *Arabidopsis thaliana*, and that EMS-induced mutations in eIF4E and eIF4G inhibited accumulation and cell-to-cell movement of CMV (Yoshii et al., 2004). One hypothesis that arises in this context is whether the mutations already identified in *PveIF4E* play a role in reducing accumulation or movement of other viruses that infect bean, especially BYMV and CMV. This is particularly relevant given that CMV is the most prevalent virus causing losses to snap bean production in the Great Lakes Region.

EMS mutagenesis within a TILLING approach (Targeting Induced Local

Lesions IN Genomes) (McCallum et al., 2000) may provide a valuable reverse genetics strategy to complement the forward search for and elucidation of natural genetic variation for virus resistance in common bean. In addition to the EMS-induced mutations in *A. thaliana* that conferred partial resistance to CMV, EMS-induced mutations were detected in eIF4E in a tomato (*Solanum lycopersicum* L.) TILLING platform that lead to the identification of a novel allele that conferred immunity to some strains and species of potyviruses (Piron et al., 2010). A collection of 5000 EMS-induced mutant lines is already available as a TILLING platform for common bean (Porch et al., 2009) and would be directly applicable to examine functional conferral, as well as for creating novel virus resistance alleles for multiple pathosystems.

ClYVV *resistance in common bean*

The research presented in Chapter 3 provided additional evidence for the role of the A227C and A332G non-synonymous SNPs in resistance to ClYVV and BCMV/BCMNV resistance. Allele-specific molecular markers detected the correct allele and predicted the correct resistance spectrum in every genotype tested. These results suggest that entire germplasm collections could be screened for the presence of these specific mutations in *PveIF4E* with the allele-specific molecular markers without the costs associated with phenotyping tens of thousands of susceptible accessions. Alternatively, a technique such as high-resolution melting analysis (HRM) could be employed to identify novel mutations in *PveIF4E* or other host translation factors (Hofinger et al., 2009; Hoffinger et al., 2011). A combination of phenotyping,

allele-specific markers, and/or HRM should be considered to identify additional *PveIF4E* haplotypes in wild and or landrace common bean germplasm to provide a better understanding of the genetic diversity, selection history, and phylogenetic relationships of this important mechanism for resistance to viruses. The evaluation of the core collection and the extended host differential panel, as well as previous research (Morales, 2006) suggest that germplasm of Middle American origin, and particularly genotypes belonging to Race Durango harbor the most concentrated source of allelic diversity for virus resistance, and perhaps extensive screening of this material is justified if it can be assembled. The characterization of entire germplasm collections is likely a future research objective when common bean germplasm collections will be characterized in a genome-wide fashion as is currently being carried out in maize (*Zea mays* L.) at the International Maize and Wheat Improvement Center (CIMMYT) (Semagn et al., 2013).

The germplasm evaluation established that additional resistance alleles to ClYVV at loci independent of *Bc-3* deserve additional attention. A revised model of resistance to ClYVV was suggested that includes the *bc-u*, *bc-2*^{2} allele combination for resistance to BCMV/BCMNV and the *By-2* allele for resistance to BYMV. The *bc-u*, *bc-2*^{2} allele combination provides effective resistance to all pathogroups of BCMV and BCMNV except for pathogroup VII (NL 4, US 6), and provides hypersensitive resistance to all pathogroups when the *I* allele is present (Drijfhout and Morales, 2005). Despite the wide virus resistance spectrum conferred by this allele combination, the *Bc-2* locus has never been located on the core genetic map of common bean. As it may be desirable to pyramid this allele combination with the *bc-3*

or *bc-3*^{2} resistance allele for more durable resistance to ClYVV, or to examine the potential of an enhanced resistance spectrum when in combination, allele-specific molecular markers would be necessary to identify the hypostatic *bc-u*, *bc-2*^{2} combination in the presence of the epistatic *bc-3*$^{(2)}$ allele. Linkage mapping populations were developed by crossing IVT 7233 x Hystyle and are available for future use. Fortunately, powerful new tools are available for molecular mapping and marker development to achieve this objective (Chapter 4).

The heterogeneous responses in common bean genotypes that possessed the *bc-u*, *bc-2*^{2} combination, where all plants exhibited necrotic local lesions, and some individual plants perished due to a delayed systemic necrosis needs additional examination. Enzyme-linked immunosorbent assay (ELISA)-based ClYVV detection failed to detect the virus in the primary leaves suggesting a resistance response, but was able to detect the virus in later developmental stages in tissue that exhibited the necrosis response. This suggests that a variant of ClYVV may have arisen and overcome the resistance. Recovery of a virus isolate from tissue undergoing the necrosis response, reinfection, and establishment of a homogeneous response in a range of common bean genotypes could confirm this hypothesis. Examination of the interaction of this response with temperature would also be worthwhile. If a resistance breaking isolate can be established, additional research into the molecular determinant for the restoration of virulence and the corresponding host avirulence protein could be investigated. Molecular mapping and development of allele-specific markers would be particularly valuable to confirm the presence and dosage of the *bc-u* and *bc-2*^{2} resistance alleles, and an examination of the effect of varying temperatures on the

frequency of resistance breaking would also be informative.

By-2 resistance to BYMV *and* ClYVV

The research presented in Chapter 4 adapted GBS based on the common bean reference genome to localize the *By-2* allele to a relatively narrow physical position in the common bean genome on chromosome 2. GBS in common bean with *ApeKI* based complexity reduction proved to be extremely useful for the purposes in which it was applied. Future use of GBS in common bean will need to strike a balance between the expected sequencing coverage and the number of SNPs estimated to be needed for the biological questions to be answered. In this sense, considerable additional research on the adaptation of GBS to common bean should be carried out and should include the evaluation of additional enzymes, as well as alternative alignment, SNP calling, and imputation algorithms to establish the most useful approaches to applications such as QTL and linkage mapping, GWAS, and genomic selection.

Although the near isogenic lines B-21 and BT-1 did not improve the results of the association analysis, their presence in the sequencing library allowed for a total of 144 SNPs to be discovered and genotyped within the physical region, and allowed for further haplotype analysis in the region to discover associations that may not have been deemed significant by the statistical model employed in the GWAS. These SNPs will provide the polymorphism needed for fine mapping and cloning the *By-2* allele. Examination of fine-scale recombination in the region based on the unimputed data suggests the potential for an even smaller physical position. Fine mapping and cloning of *By-2* will only be possible if enough genetic recombination can be incorporated into

the experimental material to be used for this effort, and this may be challenging given that at least part of this region has exhibited suppression of recombination (Vallejos et al., 2006). While this is a challenging proposition, the limitations are no longer due to a lack of information or a lack of effective and cost efficient techniques to survey SNP-based genetic variation, rather the limitation in all future allele discovery efforts will be recombination in the region(s) of interest.

Additional in-depth research into the nature of the resistance response that is conditioned by *By-2* as well as the delayed systemic necrosis phenotype is needed. The *By-2* allele resides in a physical position that contains the *I* allele for resistance to BCMV. The *I* allele has been demonstrated to be an incompletely dominant allele where the result of interactions with BCMV strains is dependent on allele dosage, temperature, virus strain, and plant cultivar (i.e. genetic background) (Collmer et al., 2000). When bean genotypes that posses the *I* allele in the homozygous or heterozygous state are inoculated with BCMV, delayed systemic necrosis was observed in both genotypes, but only under high temperatures (34°C). This interaction is in contrast to the evidence for low-temperature sensitivity of the *By-2* allele presented in Chapter 4. In terms of genetic background, the one genotype that possessed the *bc-3*^{2}, *By-2* allele combination was not heterogeneous for the delayed systemic necrosis response to ClYVV (Chapter 3), suggesting that *bc-3*^{2} is epistatic to *By-2*. This genotype, and a collection of other similar genotypes should be created to examine if the presence of *bc-3*^{2} or *bc-3* in *By-2* genotypes can also prevent the delayed systemic necrosis response. It is clear that several important factors influence the outcome of *By-2* BYMV/ClYVV interactions, and these factors need to be

investigated in greater depth. Field based testing and observation of *By-2* genotypes is imperative before any deployment of this allele takes place because of the potential for delayed systemic necrosis in response to BYMV.

Finally, two genotypes, UI 31 GN (*i*, *bc-u*, *bc-1*^{2}, *bc-2*^{2}, *bc-3*^{2}) and Monroe (*i*, *bc-u*, *bc-2*^{2}) were resistant to ClYVV-NY and BYMV-NY, and none of the individuals of these lines displayed delayed systemic necrosis in response to BYMV-NY. These lines do not possess *By-2*, and represent an additional source/mechanism of BYMV resistance. Previous research provided evidence that UI 31 GN possessed 2 to 3 complementary recessive alleles that conditioned resistance to BYMV. The inheritance and molecular basis for this resistance should be examined, and much wider survey of the genetic diversity for BYMV resistance in common bean should be undertaken in the future.

Developing multiple-virus-resistant snap beans

This research has developed important knowledge, tools, and additional hypotheses for the identification, introgression, and pyramiding of natural virus resistance alleles that could enhance the sustainability of snap bean production in the United States and around the world. Additional research into the inheritance and molecular basis for partial and complete resistance phenotypes to other component viruses is needed, especially with regards to CMV. Given the long evolutionary history of interactions between plants and viruses and the new wealth of genomic information, comparative virology and comparative genomic analyses of virus resistance in other plants (e.g. *A. thaliana*) and particularly in important legumes such

as soybean (*Glycine max* L. (Merr.)) and the model species *Medicago truncatula* Gaertn. may prove particularly worthwhile in developing a global understanding of virus resistance across all legumes. Molecular markers, particularly those based on functional polymorphisms, and including those developed here, will be necessary to better track and evaluate target genotype resistance alleles, allele combinations, and to identify and reduce linkage drag intervals to select the most elite genotypes. This research has enabled efficient marker-assisted pyramiding of the *bc-3* and *By-2* resistance alleles to ClYVV and for selection and introgression of *By-2* for BYMV resistance in the context of snap bean improvement. This directly contributes to the effort to achieve the most effective and durable resistance to the virus complex impacting U.S. Great Lakes snap bean production.

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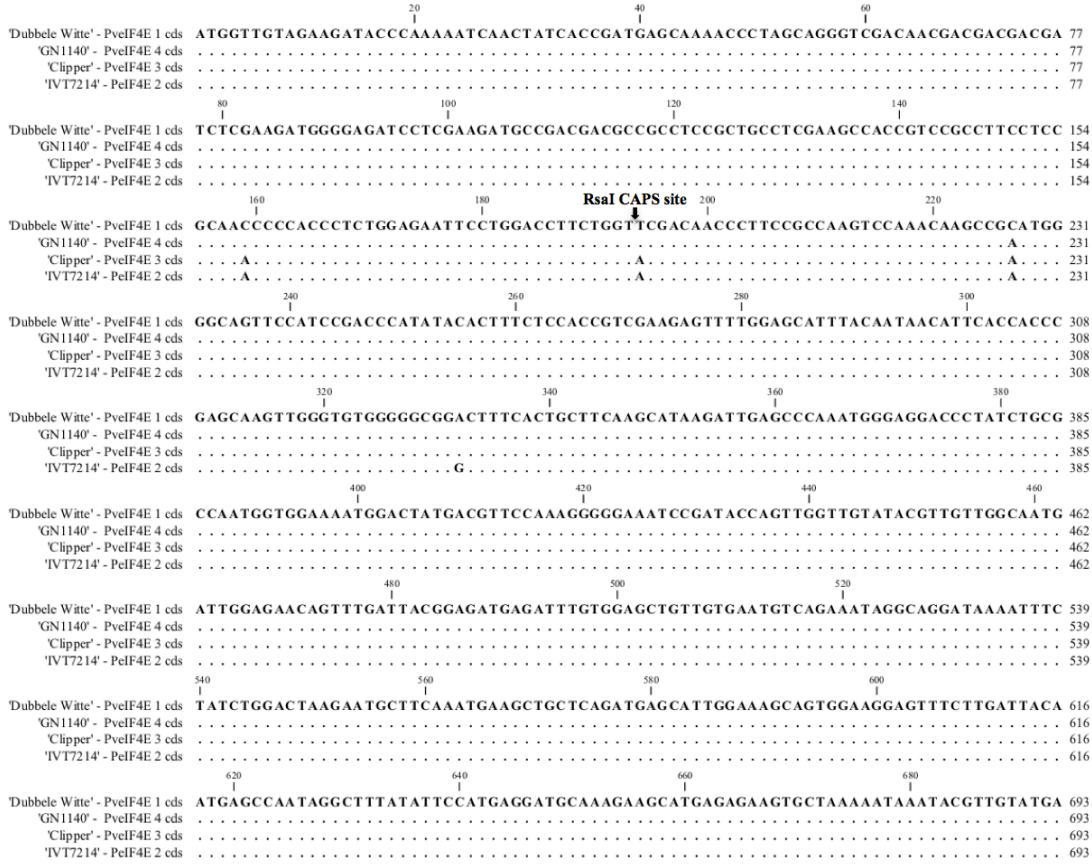
APPENDICES

Appendix 2.1. Reaction of BCMV/BCMNV common bean host differential groups and genotypes to ClYVV-NY.

Host Group	Host Differential[a]	Resistance genes	Symptoms†		
			Primary 10 dpi	Secondary 20 dpi	Secondary 50 dpi
0	Dubbele Witte	None	VN	sS, VN, sM, TN	TN/D
1	Stringless Green Refugee	*i, bc-u*	VN	sS, sM, VN	sS, sM, VN
2	Redlands Greenleaf C	*i, bc-u, bc-1*	NS	sS, sM	sS, sM
2	Puregold Wax	*i, bc-u, bc-1*	VN	sS, sM, VN	sS, sM, VN
2	Imuna	*i, bc-u, bc-1*	NS	NS	NS
3	Redlands Greenleaf B	*i, bc-u, bc-1²*	NS	sS, sM	sS, sM
3	GN UI 59	*i, bc-u, bc-1²*	NS	NS	NS
3	GN UI 123	*i, bc-u, bc-1²*	NS	NS	NS
3	GN 1140	*i, bc-u, bc-1²*	NS	NS	NS
4	Michelite 62	*i, bc-u, bc-2*	VN	VN, TN/D	TN/D
4	Sanilac	*i, bc-u, bc-2*	VN	VN, TN/D	TN/D
4	UI-34 Red Mexican	*i, bc-u, bc-2*	VN	sS, sM, VN	VN, TN/D
5	UI-114-8 Pinto	*i, bc-u, bc-1, bc-2*	NS	NS	NS
6	Othello	*i, bc-u, bc-2²*	NLL	NS	NS
6	Monroe	*i, bc-1², bc-2²*	NLL	NS	NS
7	IVT 7214	*i, bc-u, bc-2, bc-3*	NS	NS	NS
8	Black Turtle I	*I*	VN	sS, sM, VN	VN, TN/D
9	Jubila	*I, bc-1*	VN	sS, sM, VN	VN, TN/D
9	Jolanda	*I, bc-1*	NS	NS	NS
9	Top Crop	*I, bc-1*	VN	VN, TN/D	TN/D
9	Imp. Tendergreen	*I, bc-1*	VN	VN, TN/D	TN/D
10	Amanda	*I, bc-1²*	NS	NS	NS
11	92-US-1006	*I, bc-u, bc-2²*	NLL	NS	NS
11	IVT 7233	*I, bc-u, bc-1², bc-2²*	NLL	NS	NS
12	TARS-VR-1S	*I, bc-3*	NS	NS	NS

†Symptom legend: D = plant death, NS = no symptoms, NLL = necrotic local lesions, sM = severe mosaic, sS = severe stunting, VN = vein necrosis, TN = top necrosis

Appendix 2.2. Coding sequence alignment of *PveIF4E* from 4 common bean genotypes that represent the alleles investigated. The RsaI site for the previously developed† CAPS marker associated with *bc-3* resistance is indicated.

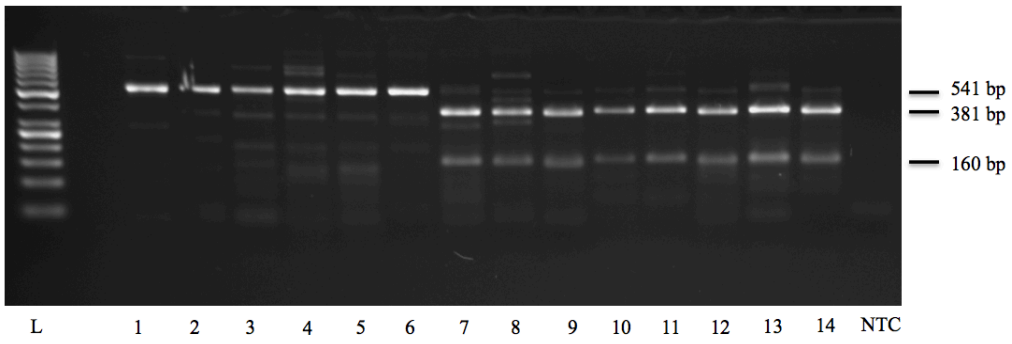


†Naderpour et al. 2010

Appendix 2.3. Amino-acid sequence alignment of PveIF4E from 4 common bean genotypes that represent the alleles investigated.

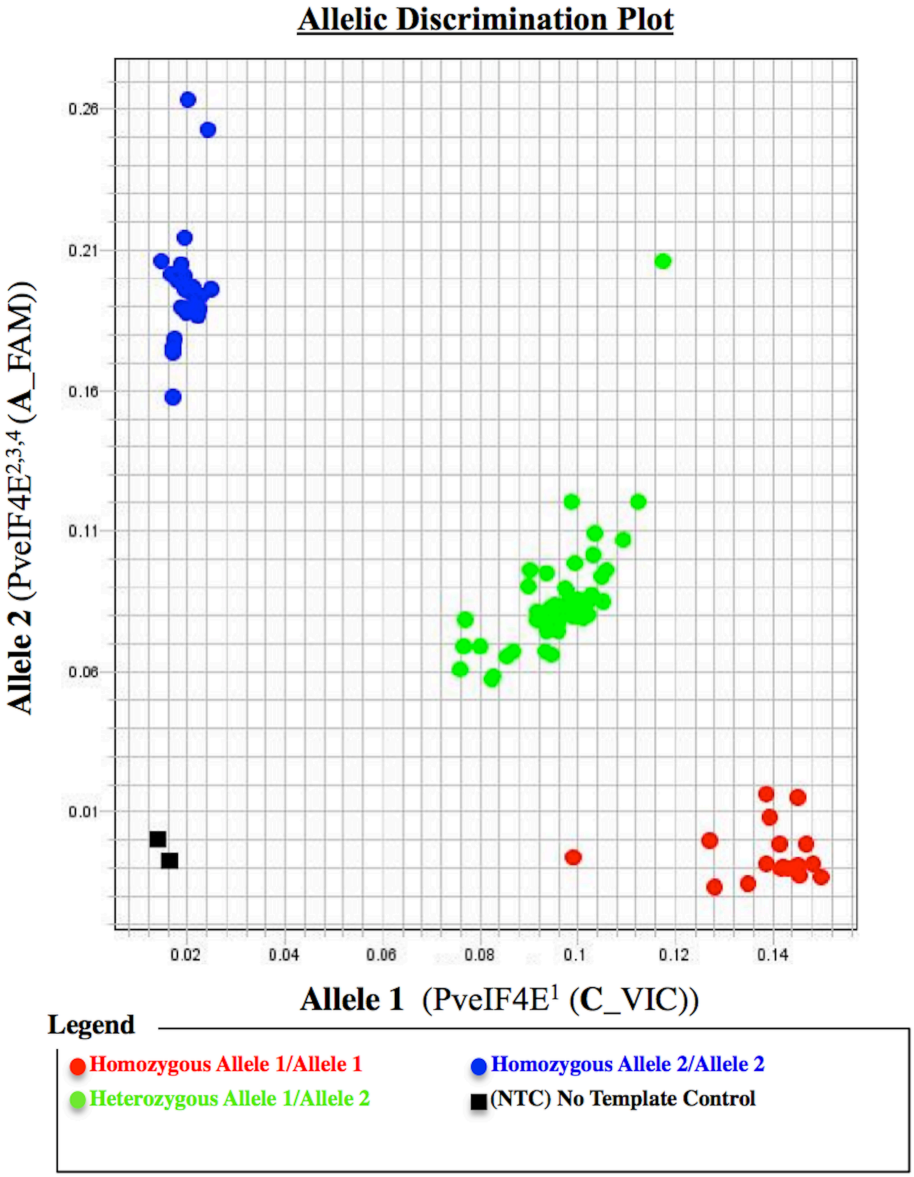
```
                                          20                  40                  60
'Dubbele Witte' - PveIF4E 1 protein MVVEDTQKSTITDEQNPSRVDNDDDDLEDGEILEDADDAASAASKPPSAFLRNPHPLENSWTFWFDNPSAKSKQAAW 77
'GN1140' - PveIF4E 4 protein        ....................................................................E. 77
'Clipper ' - PveIF4E 3 protein      ..................................K.................Y..........E. 77
'IVT7214' - PveIF4E 2 protein       ..................................K.................Y..........E. 77
                                    80                  100                 120                 140
'Dubbele Witte' - PveIF4E 1 protein GSSIRPIYTFSTVEEFWSIYNNIHHPSKLGVGADFHCFKHKIEPKWEDPICANGGKWTMTFQRGKSDTSWLYTLLAM 154
'GN1140' - PveIF4E 4 protein        ................................................................... 154
'Clipper ' - PveIF4E 3 protein      ................................................................... 154
'IVT7214' - PveIF4E 2 protein       ..................................G................................ 154
                                        160                 180                 200                 220
'Dubbele Witte' - PveIF4E 1 protein IGEQFDYGDEICGAVVNVRNRQDKISIWTKNASNEAAQMSIGKQWKEFLDYNEPIGFIFHEDAKKHERSAKNKYVV* 231
'GN1140' - PveIF4E 4 protein        ................................................................... 231
'Clipper ' - PveIF4E 3 protein      ................................................................... 231
'IVT7214' - PveIF4E 2 protein       ................................................................... 231
```

Appendix 2.4. Gel electrophoresis image of DNA fragments amplified and digested by the previously developed† RsaI CAPS marker that is associated with *bc-3* resistance. The abbreviations for each lane represent the following: L: Ladder; 1: Dubbele Witte; 2: Midnight; 3. Hystyle; 4: GN 1140, 5: Black Knight; 6: Amanda; 7: Imuna; 8: Evolutie; 9: Clipper; 10: IVT 7214; 11: Raven; 12: B/R RIL105-25, 13: USWK-6; 14: USWKH x H S_4; NTC: No template control.



† Naderpour et al. 2010

Appendix 2.5. An example of allele calls from the KASPar SNP assay PveIF4E[1]__PveIF4E[2,3,4] (**C227A**) for a subset of 88 individuals from the 'Midnight' x 'Black Knight' F_2 population segregating resistance to ClYVV-NY (Table 2.5). The resistance allele (PveIF4E[2,3,4] (**A**)) is reported by FAM (*blue*), the susceptible allele (PveIF4E[1] (**C**)) is reported by VIC (*red*) and the heterozygotes are represented in *green*



Appendix 3.1. *P. vulgaris* core collection accessions, designation, origin, and response to ClYVV-NY. Resistant accessions are shaded in grey.

Accession	Designation	Origin†	ClYVV-NY‡
PI 151407	No. 31	SA	R
PI 181996	G684	CA	S
PI 182000	No. 5	CA	S
PI 182004	No. 9	CA	S
PI 189016	Kak tsin ubul	CA	S
PI 189407	Rey Mundo	CA	S
PI 190078	O'ez Tsinap'ul	CA	S
PI 194574	G778	CA	S
PI 195402	No. 2777	CA	S
PI 195801	No. 2803	CA	S
PI 196463	No. 2939	CA	S
PI 197031	G803	CA	S
PI 200956	G826	CA	S
PI 200967	G18756	CA	S
PI 201004	G1256	CA	S
PI 201010	No. 3338	CA	S
PI 206223	G1286	CA	S
PI 208774	G949	CA	S
PI 209479	G951	CA	S
PI 209482	G16837	CA	S
PI 209486	G1361	CA	S
PI 209491	G18800	CA	S
PI 209498	G1363	CA	S
PI 288016	Negro Nicaraguense	CA	S
PI 297295	Antioquia 6	CA	S
PI 304110	Hondureno Blanco	CA	S
PI 304113	G18907	CA	S
PI 307788	S-219-R	CA	S
PI 307790	G1724	CA	S
PI 307808	G2182	CA	S
PI 307810	G1734	CA	S
PI 307816	G2184	CA	S
PI 307820	G1739	CA	S
PI 307823	S-300-R	CA	S
PI 308898	Line 7	CA	S
PI 308908	Criollo blanco No. 2	CA	S
PI 309823	Col. No. 23, sel. #3	CA	S
PI 309825	Col. No. 23, sel. #5	CA	S
PI 309827	Col. No. 23, sel. #7	CA	S
PI 309830	Col. No. 23, sel. #10	CA	S
PI 309837	Frijo chileno y colorado	CA	S
PI 309844	Frijol amarillo carne	CA	S
PI 309845	Col. No. 20670, lot #1	CA	S
PI 309857	Col. No. 20670, lot #13	CA	S
PI 309877	Col. No. 20670, lot #33	CA	S
PI 310511	Calima	CA	S
PI 310515	G1857	CA	S
PI 310546	Col. No. 21507	CA	S

Continued on next page.

Appendix 3.1 Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 310556	Maton	CA	S
PI 310561	Col. No. 21656	CA	S
PI 310586	Sangre de Toro	CA	S
PI 310599	Balin	CA	S
PI 310660	G1946	CA	S
PI 310663	G16286	CA	S
PI 310668	G1951	CA	S
PI 310674	G1957	CA	S
PI 310690	G1970	CA	S
PI 310718	G1989	CA	S
PI 310726	Xucu mama	CA	S
PI 310739	G2005	CA	S
PI 310751	G16293	CA	S
PI 310761	G2022	CA	S
PI 310778	G2031	CA	S
PI 310786	G2035	CA	S
PI 310814	G2056	CA	S
PI 310818	G2060	CA	S
PI 310826	G2068	CA	S
PI 310828	G2070	CA	S
PI 310836	G2078	CA	S
PI 310842	G2084	CA	S
PI 310850	Col. No. 21564	CA	S
PI 310865	Cuaranteno	CA	S
PI 310883	G2120	CA	S
PI 310886	Bareno	CA	S
PI 310891	G2128	CA	S
PI 311794	Tineco rojo	CA	S
PI 311807	Frijol colorado obscura	CA	S
PI 311843	Frijol de gato	CA	S
PI 311853	Colorado del suelo	CA	S
PI 317027	Col. No. 20718	CA	S
PI 326106	Frijol Blanco de Verdura	CA	S
PI 326110	G19217	CA	S
PI 345576	N.E.P. White-1	CA	S
PI 345581	Turrialba-1	CA	S
PI 399169		CA	S
PI 406940	W-C 1586	CA	S
PI 451885		CA	S
PI 451889		CA	NG
PI 451906		CA	S
PI 451917		CA	S
PI 451921		CA	S
PI 476751	Frijol colorado	CA	S
PI 533545	G9850	CA	S
PI 533561	G9877	CA	S
PI 150957	Negro	MX	S
PI 165422	G167	MX	S
PI 165423	G168	MX	S

Continued on next page.

Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 165462	AMUSGO	MX	S
PI 165466	No. 1097	MX	S
PI 201296	G8894	MX	S
PI 201324	G18762	MX	S
PI 201329	M 3194	MX	S
PI 201343	G18767	MX	S
PI 201360	G853	MX	S
PI 201369	G18773	MX	S
PI 201370	No. 3570	MX	S
PI 201387	G18783	MX	S
PI 201388	G858	MX	S
PI 201480	G18784	MX	S
PI 202835	G869	MX	S
PI 203920	G874	MX	S
PI 203921	No. 12149	MX	S
PI 203924	G18791	MX	S
PI 203934	G876	MX	S
PI 203936	G878	MX	S
PI 203958	Negro	MX	S
PI 224715	Agua de Leon	MX	S
PI 224718	De Vara	MX	S
PI 224728	Rosado	MX	S
PI 263593	G1039	MX	S
PI 263596	G1040	MX	S
PI 268110	Jamapa	MX	S
PI 309698	Frijol encerado	MX	S
PI 309700	Frijol apetito	MX	S
PI 309701	Frijol rosita	MX	S
PI 309715	G1766	MX	S
PI 309759	Higuerillo	MX	S
PI 309787	Frijol azufrado	MX	S
PI 309810	Frijol azufrado	MX	S
PI 310611	Frijol de bara	MX	S
PI 311900	Frijol azufrado	MX	S
PI 311940	Frijol aribenyo	MX	S
PI 311942	Frijol tinto	MX	S
PI 311944	Frijol de riego	MX	S
PI 311947	Frijol abolado	MX	S
PI 311956	Frijol criolla	MX	S
PI 311962	Frijol canario	MX	S
PI 311967	Frijol santanero	MX	S
PI 311974	Frijol delgado	MX	S
PI 311982	Brijol enredo	MX	S
PI 311999	Frijol colorado	MX	S
PI 312016	Negro de guia	MX	S
PI 312017	Frijol bayito	MX	S
PI 312018	Frijol negro bolito	MX	S
PI 312031	Frijol negro de mata	MX	S
PI 312052	Frijol colorado	MX	S

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Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 312064	Frijol de mata	MX	S
PI 312083	Frijol flor de Mayo	MX	S
PI 312090	Frijol mantequilla	MX	S
PI 312098	G2408	MX	S
PI 313217	Singuino	MX	S
PI 313237	Manzano	MX	S
PI 313254	De suelo negro	MX	S
PI 313270	Barreton	MX	S
PI 313297	G8904	MX	S
PI 313322	Palacio higuerilla	MX	S
PI 313328	Negro brilliante	MX	S
PI 313333	Bayo gordo	MX	S
PI 313334	Ojo de liebre	MX	S
PI 313343	G2472	MX	S
PI 313348	Rojito	MX	S
PI 313357	Bayo delgado	MX	S
PI 313366	Cacahuate	MX	S
PI 313373	Burro bola	MX	S
PI 313386	Mezquitillo	MX	S
PI 313394	Ejote	MX	S
PI 313397	Bayo	MX	S
PI 313408	Gris	MX	S
PI 313412	Blanco	MX	S
PI 313425	Bayo cafe	MX	S
PI 313429	Morado claro	MX	S
PI 313440	Amarillo	MX	S
PI 313444	Negro brillante	MX	S
PI 313445	Negro brillante	MX	S
PI 313458	Bayo	MX	S
PI 313459	Mexicano	MX	S
PI 313470	Negro	MX	S
PI 313473	Cafe	MX	S
PI 313483	Blanco chico	MX	S
PI 313486	G19123	MX	S
PI 313487	G2494	MX	S
PI 313490	Negro brill	MX	S
PI 313495	Negro brill	MX	S
PI 313499	Boludo	MX	S
PI 313501	Parraleno colorado	MX	S
PI 313512	Amarillo	MX	S
PI 313524	Vaquita	MX	S
PI 313531	Apetito	MX	S
PI 313532	10-A-1	MX	S
PI 313535	Buengusto	MX	S
PI 313537	Panza puerca	MX	S
PI 313701	G2580	MX	S
PI 313709	G2587	MX	S
PI 313720	G2594	MX	S
PI 313727	Bayo tepetate	MX	S

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Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 313733	G16350	MX	S
PI 313749	Negro	MX	S
PI 313782	G2639	MX	S
PI 313809	Negro	MX	S
PI 313820	Col. No. 451	MX	S
PI 313830	G2676	MX	S
PI 313833	Gotero 1-58	MX	S
PI 313835	Colorado de mata	MX	S
PI 313837	G2682	MX	S
PI 313839	Rojito	MX	S
PI 317350	frijol de raton	MX	S
PI 318691	Frijol	MX	S
PI 318694	G12863	MX	S
PI 318695	G12864	MX	S
PI 318703	G12870	MX	S
PI 319554	G2774	MX	S
PI 319573	Frijol mantequilla gordo	MX	S
PI 319587	Frijol ejotero	MX	S
PI 319592	Frijol garbancillo blanco	MX	S
PI 319595	Frijol japones	MX	S
PI 319607	Frijol bayo rata	MX	S
PI 319618	Frijol flor de Mayo	MX	S
PI 319619	Frijol canario	MX	S
PI 319636	G2833	MX	S
PI 319640	G2837	MX	S
PI 319674	Frijol apetita	MX	S
PI 319677	Frijol bolito	MX	S
PI 319683	Frijol morada bolita	MX	S
PI 319684	Frijol Higuerilla	MX	S
PI 325614	G16396	MX	S
PI 325618	G16397	MX	S
PI 325626	Frijol negro grueso	MX	S
PI 325630	Pinto 168	MX	S
PI 325635	Frijol negro	MX	S
PI 325642	Frijol sauhuetoli	MX	S
PI 325653	Frijol badgito	MX	S
PI 325664	Col. No. 22524	MX	S
PI 325676	Frojol de raton	MX	S
PI 325684	G12878	MX	NG
PI 325685	G12879	MX	NG
PI 325687	Frijol del raton	MX	S
PI 325691	G12883	MX	S
PI 325722	CANARIO 102	MX	S
PI 325731	Flor de Mayo	MX	S
PI 325732	G19181	MX	S
PI 325750	G19187	MX	S
PI 346955	Silvestre 1	MX	S
PI 346960	G19255	MX	S
PI 416468	65-96-1945	MX	S

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Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 416713	G14166	MX	S
PI 417616	Flor de Mayo	MX	S
PI 417621	Frijol de Coyote	MX	S
PI 417622	G12890	MX	S
PI 417627	G12894	MX	S
PI 417628	G12895	MX	S
PI 417630	G12897	MX	S
PI 417633	G12900	MX	S
PI 417634	G12901	MX	S
PI 417641	G12905	MX	S
PI 417645	G12909	MX	S
PI 417647	M7241-C	MX	S
PI 417653	G12910	MX	S
PI 417654	G12911	MX	S
PI 417657	M7252	MX	S
PI 417679	G2919	MX	S
PI 417697	Coyote	MX	NG
PI 417707	G12943	MX	S
PI 417708	G12944	MX	S
PI 417716	Ochenteno	MX	S
PI 417721	Enredador paraleno	MX	S
PI 417725	Criollo mateado	MX	S
PI 417731	M7323-3-1-3	MX	S
PI 417739	Criollo	MX	S
PI 417742	M7336	MX	S
PI 417743	Frijol Aluvial	MX	S
PI 417754	Negro	MX	S
PI 417778	G12952	MX	S
PI 417780	G12953	MX	S
PI 417782	G12955	MX	S
PI 417784	M7409-K	MX	S
PI 417786	G12957	MX	S
PI 417790	M7425	MX	S
PI 430200	M7402A	MX	S
PI 430201	M7408S	MX	S
PI 430204	Negro	MX	S
PI 430206	Rosita	MX	S
PI 449389	Negro	MX	S
PI 449410	M 7975	MX	S
PI 449412	M 8072-C-1	MX	S
PI 512003	Frijol Histaca.	MX	S
PI 533249	M7487-3-1-Bulk	MX	S
PI 533259	M7556	MX	S
PI 533277	M7671A,B,C,D	MX	S
PI 533281	M7694	MX	S
PI 533299	M8000	MX	S
PI 533311	Rosita	MX	S
PI 533312	M8154	MX	S
PI 533313	M8164	MX	S

Continued on next page.

Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 533316	M7493	MX	S
PI 533332	Canario	MX	S
PI 533373	M8072	MX	S
PI 533420	M8005-1	MX	S
PI 533428	M8083-1 Bulk	MX	S
PI 533432	M8100	MX	S
PI 533437	M8146-1	MX	S
PI 533475	M8169B-1	MX	S
PI 533476	M8207-1-Bulk	MX	S
PI 533484	M9510A,B	MX	S
PI 533498	Flor de Mayo	MX	S
PI 533502	M9538	MX	S
PI 533510	Cacahuate	MX	S
PI 533528	M9593	MX	S
PI 152208	G107	SA	S
PI 152311	Blanco Torta	SA	S
PI 198026	G18753	SA	S
PI 198037	Plomo	SA	S
PI 207136	Chileno	SA	S
PI 207148	Estrada Rosado	SA	S
PI 207165	No. 39	SA	S
PI 207180	Feijao Pico de Oro	SA	S
PI 207182	G918	SA	S
PI 207186	Matahambre Negro	SA	S
PI 207203	B.1213	SA	S
PI 207253	Puebla 8-B	SA	S
PI 207279	Chiapas 36-3	SA	S
PI 207300	Guanajuato 43-3	SA	S
PI 207322	Hidalgo 48-A	SA	S
PI 207336	Jalisco 31-1	SA	S
PI 207373	Oaxaca 5-1	SA	S
PI 207389	Queretaro 7-5	SA	S
PI 207420	Guarzo Rojo	SA	S
PI 207428	Revoltura	SA	S
PI 207443	Matahambre	SA	S
PI 241794	G1441	SA	S
PI 260418	PV-3	SA	S
PI 269209	Negro	SA	S
PI 269210	Plomo LM 57	SA	S
PI 282016	Algarrobeno	SA	S
PI 293353	A	SA	S
PI 293355	G18895	SA	S
PI 299019	Amarillo	SA	S
PI 313571	Antioquia 6	SA	S
PI 313572	Antioquia 12	SA	S
PI 313583	Atlantico 6	SA	S
PI 313596	Cauca 33	SA	S
PI 313597	Cauca 36	SA	S
PI 313598	Cauca 38	SA	S

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Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 313608	Cundinamarca 116	SA	S
PI 313609	Cundinamarca 120	SA	S
PI 313613	Cundinamarca 146	SA	S
PI 313615	Huila 5	SA	S
PI 313630	Narino 11	SA	S
PI 313633	Narino 47	SA	S
PI 313634	Narino 50	SA	S
PI 313636	Santandar del Norte 3	SA	S
PI 313639	Tolima 17	SA	S
PI 313658	Col. No. 5	SA	S
PI 313664	Col. No. 64	SA	S
PI 313665	Col. No. 66	SA	S
PI 313671	Col. No. 90	SA	S
PI 313674	Col. No. 132	SA	S
PI 313685	Col. No. 267	SA	S
PI 313693	Col. No.	SA	S
PI 313842	Col. No. 19	SA	S
PI 313843	Col. No. 23	SA	S
PI 313850	Col. No. 143	SA	S
PI 316016	nuna type	SA	S
PI 316023	nuna type	SA	S
PI 316030	nuna type	SA	S
PI 316031	nuna type	SA	S
PI 355419	SAM 2670	SA	S
PI 387862	W-941a	SA	S
PI 387865	W-941d	SA	S
PI 387866	W-941e	SA	S
PI 415886	Blanco	SA	S
PI 415887	E8450	SA	S
PI 415900	E8465-A&B	SA	S
PI 415906	E8474-A&B	SA	S
PI 415909	Frijol Blanco Grande	SA	S
PI 415913	E8481	SA	S
PI 415936	E8507	SA	S
PI 415949	P8474-A-D	SA	S
PI 415950	G13913	SA	S
PI 415954	Poroto Blanco Grande	SA	S
PI 415955	Poroto Blanco Chico	SA	S
PI 415975	CO8530-A-D	SA	S
PI 415986	Frijol	SA	S
PI 415987	CO8549	SA	S
PI 430167	Frijol fojo	SA	S
PI 430210	P8562	SA	S
PI 510574	Puka Poroto	SA	S
PI 531862	Nunas	SA	S
PI 533363	E7893	SA	S
PI 533577	E7877-A-18	SA	S
PI 533584	E7934-1	SA	S
PI 557483	DE-3	SA	S

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Appendix 3.1. Continued.

Accession	Designation	Origin†	ClYVV-NY‡
PI 150409	Frijol de seda balla	CA	NT
PI 189408	G739	CA	NT
PI 307791	G1169	CA	NT
PI 307806	G2181	CA	NT
PI 308894	Col. No. 4	CA	NT
PI 310829	G2071	CA	NT
PI 310915	Col. No. 21638	CA	NT
PI 343950		CA	NT
PI 165455	G169	MX	NT
PI 201354	G18769	MX	NT
PI 202834	G868	MX	NT
PI 311907	Frijol sarco	MX	NT
PI 313272	Barreton	MX	NT
PI 325721	Canario 101	MX	NT
PI 417667	M7263-D-20	MX	NT
PI 449422	Negro	MX	NT
PI 476693	VF-78 Mex. 85	MX	NT
PI 533286	M7752	MX	NT
PI 535395	TARS 144	MX	NT
PI 207127	Moro	SA	NT
PI 207154	Liberano L-10	SA	NT
PI 207193	Pinck	SA	NT
PI 207207	Z-#4	SA	NT
PI 207216	Sangretoro	SA	NT
PI 290990	Diacol Nima	SA	NT
PI 290995	Canario	SA	NT
PI 306200	G18940	SA	NT
PI 313592	Boyaca 101	SA	NT
PI 313667	Col. No. 71	SA	NT
PI 313847	Col. No. 75	SA	NT
PI 511767	120	SA	NT

†Origin classification; CA = Central America; MX = Mexico; SA = South America.
‡ClYVV-NY response; R = resistant; S = susceptible; NG = failed to germinate; NT = not tested (not available).

Appendix 3.2. Snap bean cultivar accession numbers, designations, and responses to ClYVV-NY. Resistant accessions are shaded in grey.

Accession†	Cultivar	ClYVV-NY‡
PI 599026	Amanda	*bc-3*^{2} §
PI 642144	Baby Bop	*bc-3*^{2} §
W6 42706	Evolutie	*bc-3*^{2} §
PI 599003	Imuna	*bc-3*^{2} §
PI 602987	IVT 7214	*bc-3* §
PI 599029	IVT 7233	*bc-u*, *bc-2*^{2} (NLL, dSN)
G 7591	Jolanda	*bc-3*^{2} §
PI 550261	Laureat	*bc-3*^{2} §
CU	Paloma	*bc-3*^{2} §
CU	Polder	*bc-3*^{2} §
W6 28061	RH13	*bc-3*^{2}
CU	Sonesta	*bc-3*^{2} §
PI 642143	Acclaim	S
CU	Almaty	S
CU	Amy	S
PI 550134	Atlantic	S
CU	Banga	S
CU	Barrier	S
PI 538771	BBL 110	S
PI 550403	BBL 156	S
PI 661911	Beany Baby	S
PI 550043	Benton	S
PI 549926	Blue Crop	S
CU	Bogey	S
PI 549877	Bonanza	S
PI 550421	Brio	S
CU	Cadillac	S
PI 538772	Calgreen	S
CU	Cameron	S
CU	Carson	S
CU	Castano	S
W6 26686	Celtic	S
CU	Charon	S
PI 549526	Contender	S
PI550422	Crest	S
CU	Cruiser	S
CU	Cupidon	S
PI 550037	Dandy	S
PI 585237	Daytona	S
PI 377736	Dubbele Witte	S
CU	Dynasty	S
CU	Early Bird	S
CU	Erin	S
PI 537106	Espada	S
PI 550255	EZ Pick	S
PI 561588	Flevoro	S
PI 550023	Flo	S
CU	Fresh Pick	S

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Appendix 3.2. Continued.

Accession†	Cultivar	ClYVV-NY‡
CU	Galveston	S
PI 549929	Gator Green 15	S
PI 546491	Gold Mine	S
PI 550151	Hialeah	S
PI 550288	Hystyle	S
W6 26699	Igloo	S
PI 559394	Jade	S
CU	Koala	S
CU	Kylian	S
CU	Leon	S
CU	Marseille	S
CU	Matador	S
CU	Maxibel	S
W6 26707	Medinah	S
PI 661921	Mercury	S
W6 26709	Minuette	S
PI 661922	Mirada	S
CU	Molly	S
W6 26712	Nicelo	S
W6 26713	Normandie	S
PI 538026	Opus	S
CU	Orient	S
CU	Palati	S
W6 26715	Pix	S
PI 550283	Podsquad	S
W6 26717	Probe	S
PI 550051	Producer	S
W6 26718	Prosperity	S
PI 549841	Provider	S
CU	Puncher	S
PI 599004	Redlands Greenleaf B	S
PI 599000	Redlands Greenleaf C	S
W6 26719	Rhapsody	S
CU	Scorpio	S
CU	Serin	S
PI 550708	Seville	S
W6 26723	Shade	S
CU	Slenderpack	S
PI 550045	Slimgym	S
CU	Sonata	S
CU	Speedy	S
CU	Sprite	S
PI 598999	Stringless Green Refugee	S
PI 564523	Summit	S
CU	Symphony	S
CU	Teseo	S
CU	True Blue	S
CU	Venice	S
PI 550279	Venture	S

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Appendix 3.2. Continued.

Accession†	Cultivar	ClYVV-NY‡
CU	Volta	S
CU	Warrior	S

† Accession classification; PI and W6 = USDA; CU = Cornell University.
‡ClYVV-NY response; R = resistant; S = susceptible.
§ClYVV-NY response previously reported (Hart and Griffiths, 2013).

Appendix 3.3. References cited for the assignment of recessive resistance alleles reported in Table 1.

Ref†	Reference
1	Miklas, P.N., R.C. Larsen, R. Riley, and J.D. Kelly. 2000. Potential marker-assisted selection for *bc-1*^{2} resistance to bean common mosaic potyvirus in common bean. Euphytica 116:211-219.
2	Hart J.P., and P.D. Griffiths. 2013. A series of eIF4E alleles at the *Bc-3* locus are associated with recessive resistance to Clover yellow vein virus in common bean. Theor. Appl. Genet. doi:10.1007/s00122-013-2176-8
3	Forster, R.L., C.A. Strausbaugh, K. Stewart-Williams, and J.R. Myers.1994. Determination of resistance to BCMV in dry edible bean cultivars and breeding lines. Annu. Rep. Bean Improv. Coop. 37:1-8.
4	Miklas, P., S. Lambert, G. Mink, and M. Silbernagel. 1998. Many beans with *bc-3* resistance to BCMNV are susceptible to BCMV. Annu. Rep. Bean Improv. Coop. 41:33-34.
5	Miklas, P.N., and A.N. Hang. 1998. Release of cranberry dry bean germplasm lines USCR-7 and USCR-8 with resistance to bean common mosaic and necrosis viruses. Annu. Rep. Bean Improv. Coop. 41:227-228.
6	Miklas, P.N., J.S. Beaver, J.R. Steadman, M.J. Silbernagel, and G.F. Freytag. 1997. Registration of three bean common mosaic virus-resistant navy bean germplasms. Crop Sci. 37:1025.
7	Kelly, J.D., G.L. Hosfield, G.V. Varner, M.A. Uebersax, S.D. Haley, and J. Taylor. 1994. Registration of 'Raven' black bean. Crop Sci. 34:1406-1407.
8	Miklas, P.N., A.N. Hang, J.D. Kelly, C.A. Strausbaugh, and R.L. Forster. 2002. Registration of three kidney bean germplasm lines resistant to Bean Common Mosaic and Necrosis Potyviruses: USLK-2 Light Red Kidney, USDK-4 Dark Red Kidney, and USWK-6 White Kidney. Crop Sci. 42:674-675.
9	Drijfhout E., 1978. Genetic interaction between Phaseolus vulgaris and Bean common mosaic virus with implications for strain identification and breeding for resistance. Agric. Res. Rep. 872:1-98.
10	Larsen, R.C., and P.N. Miklas. 2005. Evaluation of common bean for resistance to Clover yellow vein virus. Annu. Rep. Bean Improv. Coop. 48:57-58.
11	Fouilloux G., and H. Bannerot. 1977. RH13, a four disease resistant line. Annu. Rep Bean Improv. Coop. 20:59.
12	Dwadash-Shreni, V.C., and J.R. Stavely. 1984. Comparative resistance of Phaseolus vulgaris cultivars to Clover yellow vein virus using various inoculation methods. Plant Dis. 68:555-558.
13	Rosas, J.C., J.S. Beaver, D. Escoto, C.A. Perez, A. Llano, J.C. Hernandez, and R. Araya. 2004. Registration of 'Amadeus 77' small red common bean. Crop Sci. 44:1867-1868.
14	Rosas, J.C., J.S. Beaver, S. Beebe, and A. Viana. 2004. Names of common bean varieties released in Central America and the Caribbean. Annu. Rep. Bean Improv. Coop. 47:329-330.
15	Singh, S.P., F.J. Morales, and H. Teran. 2000. Registration of bean golden mosaic resistant dry bean germplasm GMR 1 and GMR 5. Crop Sci. 40:1836.
16	Beaver, J.S., and P.N. Miklas. 1999. Registration of 'Morales' small white bean. Crop Sci. 39:1257.

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Appendix 3.3. Continued.

	USDA-AMS. 2002. Plant Variety Protection Certificate 200200275: 'Scorpio'.
18	Rosas, J.C., O.I. Varela, and J.S. Beaver. 1997. Registration of 'Tio Canela-75' small red bean Race Mesoamerica. Crop Sci. 37:1391.
19	Beaver, J.S., M. Zapata, and P.N. Miklas. Registration of PR9443-4 dry bean germplasm resistant to bean golden mosaic, common bacterial blight, and rust. Crop Sci. 39:1262.
20	Provvidenti, R., B. Scully, D.E. Halseth, and D.H. Wallace. 1989. B-21: a dry black bean breeding line with multiple virus resistance. HortScience. 24:1049.
21	Scully, B., R. Provvidenti, D. Benscher, D.E. Halseth, J.C. Miller, and D.H. Wallace. 1995. Five multiple-virus-resistant common bean breeding lines. HortScience. 30:1320-1323.

†Reference number as it corresponds with Table 1.

Appendix 4.1. Accession, designation, BCMV/BCMNV host group (HG), corresponding resistance alleles, and visual phenotypic response to BYMV-NY and ClYVV-NY ten days post inoculation (dpi).

				BYMV-NY§	ClYVV-NY§
Accession†	Designation ‡	HG	Resistance Alleles	10 dpi	10 dpi
PI 598993	Black Turtle 2	0	None	sS, Ld, Y	sS,VN
PI 377736	Dubbele Witte	0	None	sS, Ld, Y	sS,VN
PI 598999	Str.Green Ref.	1	*i, bc-u*	sS, Ld, Y	sS,VN
PI 599003	Imuna	2	*i, bc-u, bc-1, bc-3^2*	mM	NS
PI 549559	Puregold Wax	2	*i, bc-u, bc-1*	S, Ld, M	sS,VN
PI 599000	Redlands Grnlf. C	2	*i, bc-u, bc-1*	mS, M	S, M
W6 28057	GN 1140	3	*i, bc-u, bc-1^2, bc-3^2*	mS, M	NS
PI 599006	GN UI 123	3	*i, bc-u, bc-1^2, bc-3^2*	mM	NS
PI 599004	Redlands Grnlf. B	3	*i, bc-u, bc-1^2*	mS, M	S, M
PI 599009	Michelite 62	4	*i, bc-u, bc-2*	mS, mM	sS, VN
PI 549732	RedMex. UI 34	4	*i, bc-u, bc-2*	mS, M	sS, VN
PI 549695	Sanilac	4	*i, bc-u, bc-2*	S, Ld, M	sS, VN
PI 599014	Pinto UI 114-8	5	*i, bc-u, bc-1, bc-2, bc-3^2*	mM	NS
PI 550129	Fiesta	6	*i, bc-u, bc-2^2*	sS, Ld, Y	NLL (dSN)
W6 28060	GN UI 31	6	*i, bc-u, bc-1^2, bc-2^2, bc-3^2*	NS	NS
PI 599016	Monroe	6	*i, bc-u, bc-1^2, bc-2^2*	NS	NLL (dSN)
PI 549733	Red Mex. UI 35	6	*i, bc-u, bc-2^2*	mS, Ld, mM,	NLL (dSN)
PI 602987	IVT 7214	7	*i, bc-u, bc-2, bc-3*	mM	NS
W6 36148	Don Timoteo	7	*i, bc-3*	S, Ld, M	NS
PI 599021	Black Turtle I	8	*I*	sS, Ld, Y	sS, VN
PI 550288	Hystyle	-	*I, ?*	sS, AD	sS, VN
PI 599024	Imp. Tendergreen	9	*I, bc-1*	sS, AD	sS, VN
G 7591	Jolanda	9	*I, bc-1, bc-3^2*	S, Ld, M	NS
PI 599023	Jubila	9	*I, bc-1*	sS, Ld, Y	sS, VN
PI 599025	Top Crop	9	*I, bc-1*	sS, AD	sS, VN
PI 599026	Amanda	10	*I, bc-1^2, bc-3^2*	S, Ld, M	NS
CU	Tapia	10	*I, bc-1^2*	M	S, M
PI 599029	IVT 7233	11	*I, bc-u, bc-1^2, bc-2^2*	S, Ld, M	NLL (dSN)
PI 599030	92-US-1006	11	*I, bc-u, bc-2^2*	sS, Ld, Y	NLL (dSN)
PI 594325	TARS-VR-1S	12	*I, bc-3*	mM	NS
PI 618811	USCR-7	12	*I, bc-3*	mM	NS
PI 618814	USDK-4	12	*I, bc-3*	mM	NS
PI 602988	USLK-2	12	*I, bc-3*	mM	NS
PI 618815	USWK-6	12	*I, bc-3*	mM	NS
CU	B-21	-	*I, By-2*	NS (dSN)	NS (dSN)
CU	B28S2C	-	*I, By-2*	NS (dSN)	NS (dSN)
CU	SP 17B	-	*By-2, bc-3^2*	NS	NS

† Accession classification; PI and W6 = USDA; G = CIAT; CU = Cornell University.

‡ Designation abbreviations; Str. Grn. Ref. = Stringless Green Refugee; Grnlf = Greenleaf; RedMex. = Red Mexican.

§ Phenotypic response based on nine infected plants in each of two separate experiments; sS = severe stunting; Ld = leaf deformation; Y = yellowing; mM = mild mosaic; M = mosaic; NS = no symptoms; S = stunting; AD = arrested development; NS(dSN) = heterogeneous reaction where all plants were initially symptomless , and some individual plants later exhibited delayed systemic necrosis (dSN); VN = vein necrosis on inoculated primary leaves; NLL = necrotic local lesions.

Appendix 4.2. Common adapter, barcode adapter, and PCR Primer oligonucleotide sequences.

	Oligonucleotide Sequences
Common Adapters	5'-CWGAGATCGGAAGAGCGGTTCAG CAGGAATGCCGAG-3' 5'-CTCGGCATTCCTGCTGAACCGCTCTTCCGATCT-3'
*Ape*KI Barcode Adapters†	5'-CWGxxxxAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3' 5'-ACACTCTTTCCCTACACGACGCTCTTCCGATCTyyyy-3'
PCR Primers	5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG CTCTTCCGATCT-3' 5'-CAAGCAGAAGACGGCATACGAGATCGGTCTCGGCATTCCTGCTGAA CCGCTCTTCCGATCT-3'

† *Ape*KI Barcode adapters where 'xxxx' and 'yyyy' represent the barcode, and barcode compliment respectively as they correspond the key file in Supplemental Table S3.

Appendix 4.3. The *ApeKI* barcode adapter sequences assigned to each sample are presented in this key file.

Flowcell	Lane	Barcode	Sample	Plate	Row	Column
H0DRFADXX	L002	AGGC	F5-6-1	B28HF5	A	1
H0DRFADXX	L002	GATT	F5-7-2	B28HF5	A	2
H0DRFADXX	L002	ACCGT	F5-8-3	B28HF5	A	3
H0DRFADXX	L002	CGTCA	F5-11-4	B28HF5	A	4
H0DRFADXX	L002	TCGCA	F5-12-5	B28HF5	A	5
H0DRFADXX	L002	CGCAT	F5-16-6	B28HF5	A	6
H0DRFADXX	L002	TCATAGT	F5-27-7	B28HF5	A	7
H0DRFADXX	L002	TTACGAT	F5-32-1	B28HF5	A	8
H0DRFADXX	L002	GGCTAGA	F5-35-2	B28HF5	A	9
H0DRFADXX	L002	ACAATGGA	F5-36-3	B28HF5	A	10
H0DRFADXX	L002	ACAAGAGT	F5-39-4	B28HF5	A	11
H0DRFADXX	L002	GAACATGA	Hy-2	B28HF5	A	12
H0DRFADXX	L002	AGCATT	F5-6-2	B28HF5	B	1
H0DRFADXX	L002	CTCCGA	F5-7-3	B28HF5	B	2
H0DRFADXX	L002	TTGGCA	F5-8-4	B28HF5	B	3
H0DRFADXX	L002	CCACGT	F5-11-5	B28HF5	B	4
H0DRFADXX	L002	GATGTC	F5-12-6	B28HF5	B	5
H0DRFADXX	L002	TGTTAC	F5-16-7	B28HF5	B	6
H0DRFADXX	L002	CAGTTA	F5-30-1	B28HF5	B	7
H0DRFADXX	L002	GCCTAT	F5-32-2	B28HF5	B	8
H0DRFADXX	L002	AGTGGC	F5-35-3	B28HF5	B	9
H0DRFADXX	L002	TGACCT	F5-36-4	B28HF5	B	10
H0DRFADXX	L002	TTGCAC	F5-39-5	B28HF5	B	11
H0DRFADXX	L002	CTAGCT	Hy-3	B28HF5	B	12

Continued on next page.

Appendix 4.3. continued.

Flowcell	Lane	Barcode	Sample	Plate	Row	Column
H0DRFADXX	L002	AATCGTT	F5-6-3	B28HF5	C	1
H0DRFADXX	L002	CTATGGA	F5-7-4	B28HF5	C	2
H0DRFADXX	L002	TACGGTA	F5-8-5	B28HF5	C	3
H0DRFADXX	L002	ACTATGT	F5-11-6	B28HF5	C	4
H0DRFADXX	L002	CGTGAAT	F5-12-7	B28HF5	C	5
H0DRFADXX	L002	TTGCAGA	F5-27-1	B28HF5	C	6
H0DRFADXX	L002	AACTTGT	F5-30-2	B28HF5	C	7
H0DRFADXX	L002	TGACGTA	F5-32-3	B28HF5	C	8
H0DRFADXX	L002	GCTATAA	F5-35-4	B28HF5	C	9
H0DRFADXX	L002	ATCGTAT	F5-36-5	B28HF5	C	10
H0DRFADXX	L002	TACTGAT	F5-39-6	B28HF5	C	11
H0DRFADXX	L002	CTTGAGA	Tapia-1	B28HF5	C	12
H0DRFADXX	L002	TCAAGTT	F5-6-4	B28HF5	D	1
H0DRFADXX	L002	GATCATA	F5-7-5	B28HF5	D	2
H0DRFADXX	L002	GCATTGA	F5-8-6	B28HF5	D	3
H0DRFADXX	L002	CAGGTAT	F5-11-7	B28HF5	D	4
H0DRFADXX	L002	TGCAATA	F5-16-1	B28HF5	D	5
H0DRFADXX	L002	ATATCGT	F5-27-2	B28HF5	D	6
H0DRFADXX	L002	AGTCTAT	F5-30-3	B28HF5	D	7
H0DRFADXX	L002	GTCTGAA	F5-32-4	B28HF5	D	8
H0DRFADXX	L002	ATCAGTT	F5-35-5	B28HF5	D	9
H0DRFADXX	L002	CAGTTGA	F5-36-6	B28HF5	D	10
H0DRFADXX	L002	TGTGCAA	F5-39-7	B28HF5	D	11
H0DRFADXX	L002	CGACAGT	Tapia-2	B28HF5	D	12
H0DRFADXX	L002	ACGTGTA	F5-6-5	B28HF5	E	1
H0DRFADXX	L002	GATGCAT	F5-7-6	B28HF5	E	2
H0DRFADXX	L002	CTAATGT	F5-8-7	B28HF5	E	3
H0DRFADXX	L002	GTCGATA	F5-12-1	B28HF5	E	4
H0DRFADXX	L002	TATACGT	F5-16-2	B28HF5	E	5
H0DRFADXX	L002	GCGTAAT	F5-27-3	B28HF5	E	6
H0DRFADXX	L002	AGCGTTA	F5-30-4	B28HF5	E	7
H0DRFADXX	L002	ATCCGGA	F5-32-5	B28HF5	E	8
H0DRFADXX	L002	TCAGTAT	F5-35-6	B28HF5	E	9
H0DRFADXX	L002	CAATGTT	F5-36-7	B28HF5	E	10
H0DRFADXX	L002	GTTACGA	B21-1	B28HF5	E	11
H0DRFADXX	L002	TGCATAT	Tapia-3	B28HF5	E	12

Continued on next page.

Appendix 4.3. continued.

Flowcell	Lane	Barcode	Sample	Plate	Row	Column
H0DRFADXX	L002	CAAGAAGT	F5-6-6	B28HF5	F	1
H0DRFADXX	L002	GTCATGGT	F5-7-7	B28HF5	F	2
H0DRFADXX	L002	AACAGTGA	F5-11-1	B28HF5	F	3
H0DRFADXX	L002	GTGCAAGA	F5-12-2	B28HF5	F	4
H0DRFADXX	L002	CAATAGGA	F5-16-3	B28HF5	F	5
H0DRFADXX	L002	TGCAGTGT	F5-27-4	B28HF5	F	6
H0DRFADXX	L002	AGGCTAGA	F5-30-5	B28HF5	F	7
H0DRFADXX	L002	CTAGTGGT	F5-32-6	B28HF5	F	8
H0DRFADXX	L002	GCTAGTGT	F5-35-7	B28HF5	F	9
H0DRFADXX	L002	AGTTGGCA	F5-39-1	B28HF5	F	10
H0DRFADXX	L002	TCGCAAGT	B21-2	B28HF5	F	11
H0DRFADXX	L002	CGATGTGT	BT-1-1	B28HF5	F	12
H0DRFADXX	L002	AACGTAGA	F5-6-7	B28HF5	G	1
H0DRFADXX	L002	CTCACGGA	F5-8-1	B28HF5	G	2
H0DRFADXX	L002	TAGCGTGT	F5-11-2	B28HF5	G	3
H0DRFADXX	L002	ACGTAAGA	F5-12-3	B28HF5	G	4
H0DRFADXX	L002	CGTATGGT	F5-16-4	B28HF5	G	5
H0DRFADXX	L002	GTACGTGT	F5-27-5	B28HF5	G	6
H0DRFADXX	L002	TTCGAAGA	F5-30-6	B28HF5	G	7
H0DRFADXX	L002	AATACGGA	F5-32-7	B28HF5	G	8
H0DRFADXX	L002	TGACTGGT	F5-36-1	B28HF5	G	9
H0DRFADXX	L002	GCGGATGT	F5-39-2	B28HF5	G	10
H0DRFADXX	L002	CATTGAGA	B21-3	B28HF5	G	11
H0DRFADXX	L002	GTAACAGA	BT-1-2	B28HF5	G	12
H0DRFADXX	L002	AGCTTGGT	F5-7-1	B28HF5	H	1
H0DRFADXX	L002	ACAGATGA	F5-8-2	B28HF5	H	2
H0DRFADXX	L002	CAGTTGGT	F5-11-3	B28HF5	H	3
H0DRFADXX	L002	TGCAAGAA	F5-12-4	B28HF5	H	4
H0DRFADXX	L002	ACTCGAGA	F5-16-5	B28HF5	H	5
H0DRFADXX	L002	GGAGCTGT	F5-27-6	B28HF5	H	6
H0DRFADXX	L002	CTGAGTGT	F5-30-7	B28HF5	H	7
H0DRFADXX	L002	GATCAGAA	F5-35-1	B28HF5	H	8
H0DRFADXX	L002	TGCATAGA	F5-36-2	B28HF5	H	9
H0DRFADXX	L002	GAACGAAT	F5-39-3	B28HF5	H	10
H0DRFADXX	L002	TTGGCGGA	Hy-1	B28HF5	H	11
H0DRFADXX	L002	CGCCGCAT	BT-1-3	B28HF5	H	12

Appendix 4.4. TASSEL 3.0 GBS Pipeline commands and corresponding input arguments used to create the GBS dataset and hapmap (.hmp.txt) output files. If the argument is not included in the table, it was either an input command for a specific file, or the TASSEL 3.0 default was invoked.

Command	Arguments
FastqToTagCountPlugin	-e ApeKI
MergeMultipleTagCountPlugin	-t
bwa index	
bwa aln	-t 4
bwa samse	
SAMConverterPlugin	
FastqToTBTPlugin	-e ApeKI -c 5 -y
MergeTagsByTaxaFilesPlugin	
TagsToSNPByAlignmentPlugin	-mnF 0.9 -sC 1 -eC 11 -y
MergeDuplicateSNPsPlugin	-sC 1 -eC 11
GBSHapMapFiltersPlugin	-mnTCov 0.1-mnScov 0.1
MergeIdenticalTaxaPlugin	-xHets -sC 1 -eC 11

Appendix 4.5. KASP assays employed for cosegregation analysis in the B28S2C x Hystyle F_2 population.

KASP Assay ID	SNP	Primer	Sequence
Pv02_48722161	T/C	Hystyle Allele (T_FAM)	GATTCAATTGATGGTTTCTTTAAATATTCCTTA
		B28S2C Allele (C_HEX)	CAATTGATGGTTTCTTTAAATATTCCTTG
		Common	AAAGAATCCGGTGTGAGTGGCCTT
Pv02_48790627	G/T	Hystyle Allele (G_FAM)	GTACAACCTGCCCATCTCCATC
		B28S2C Allele (T_HEX)	GGTACAACCTGCCCATCTCCATA
		Common	AGCTGCATTTTCCGGATCCCTTGAA
Pv02_48843877	G/A	Hystyle Allele (G_FAM)	GCAACAAGGGGAAGTTTTTCTGTAC
		B28S2C Allele (A_HEX)	AGCAACAAGGGGAAGTTTTTCTGTAT
		Common	CACGATATGGCCATGACTGTCAACAT
Pv02_48849943	A/G	Hystyle Allele (A_FAM)	GTCACAATATAATGCACAAGCCATGTTT
		B28S2C Allele (G_HEX)	CACAATATAATGCACAAGCCATGTTC
		Common	GCCATGCTCATGCATGTATCCAAGAT
Pv02_48874335	G/A	Hystyle Allele (G_FAM)	CAGAACATCATCCGTTTACCCAAATG
		B28S2C Allele (A_HEX)	CAGAACATCATCCGTTTACCCAAATA
		Common	GCATTTTCGTGATTGACAGTCTCAAAGTT
Pv02_48891077	G/T	Hystyle Allele (G_FAM)	GTGCGCAGCTGTGTCAGATTG
		B28S2C Allele (T_HEX)	GGTGCGCAGCTGTGTCAGATTT
		Common	CCGGCCAAATCCCTCTCGGAAT
Pv02_49012008	T/G	Hystyle Allele (T_FAM)	GAGTTAGTGGCAGTGCAGCT
		B28S2C Allele (G_HEX)	GAGTTAGTGGCAGTGCAGCG
		Common	CTTCTCTTGCTCTCACCCATCGTAA

Appendix4.6. BYMV-NY interaction phenotypes in two separate populations developed to analyze the inheritance of the *By-2* resistance response at lower temperatures.

Season†	Temp.‡	Population	Phenotype§	Number
Winter	24° day/21°C night	Hystyle	S	10
		B28S2C	R	6
			R(dSN)	4
		F2	R	61¶
			R(dSN)	88
			S	51
Winter	21° day/21°C night	BT-1	S	10
		B-21	R(dSN)	10
		F1	R(dSN)	10
		F2	R	22#
			R(dSN)	99
			S	45

† The Hystyle x B28S2C populations were inoculated in Dec. 2012 and the BT-1 x B-21 populations were inoculated in Jan. 2013

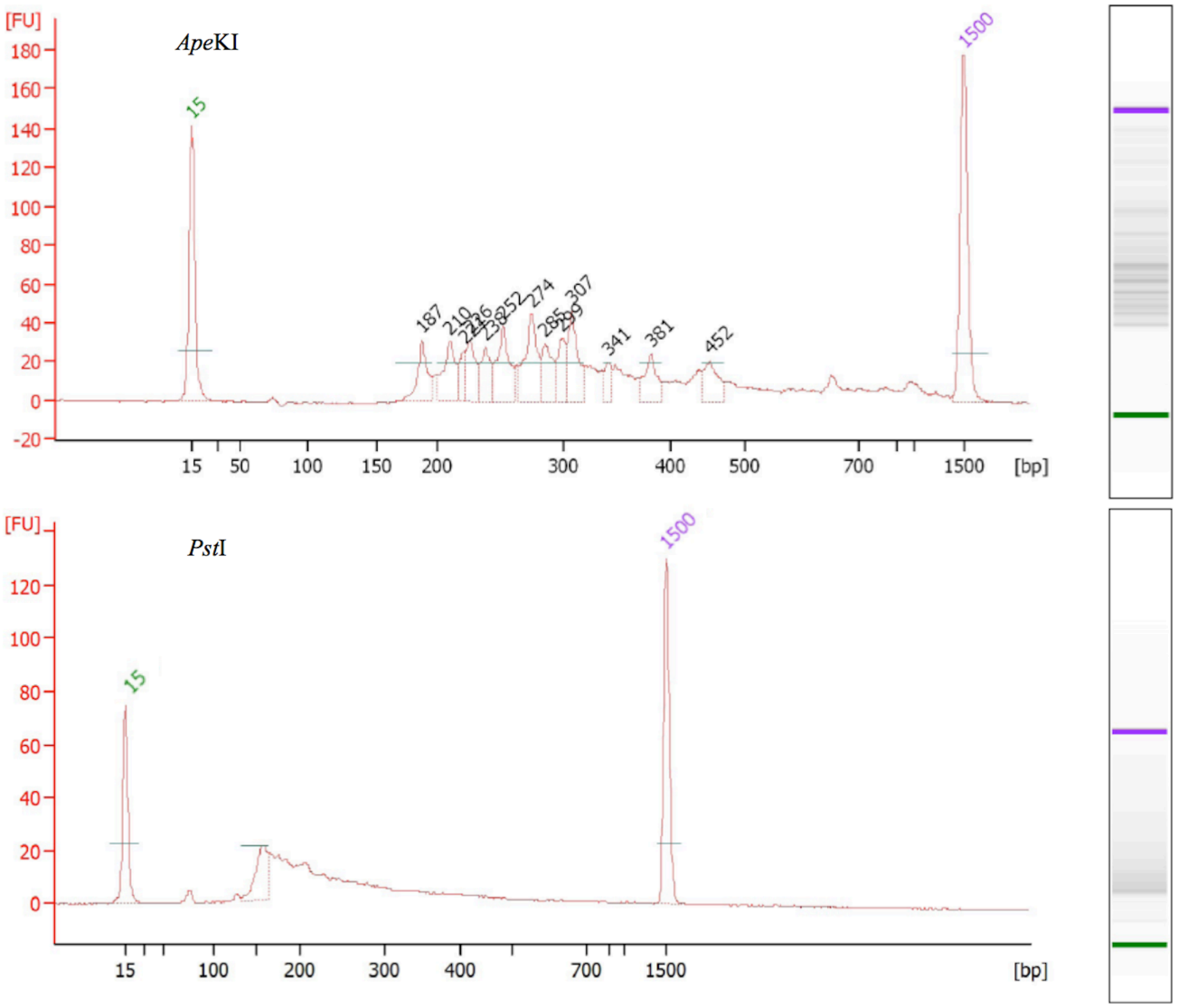
‡ Observed temperatures fluctuated 1-2°C

§ Response to infection with BYMV-NY, S = susceptible – pronounced stunting and mosaic; R = resistant – no symptoms; R(dSN) = Initially classified as resistant (R), then exhibited delayed systemic necrosis = (dSN). The phenotypes assigned were based on multiple inoculations and visual assessment only.

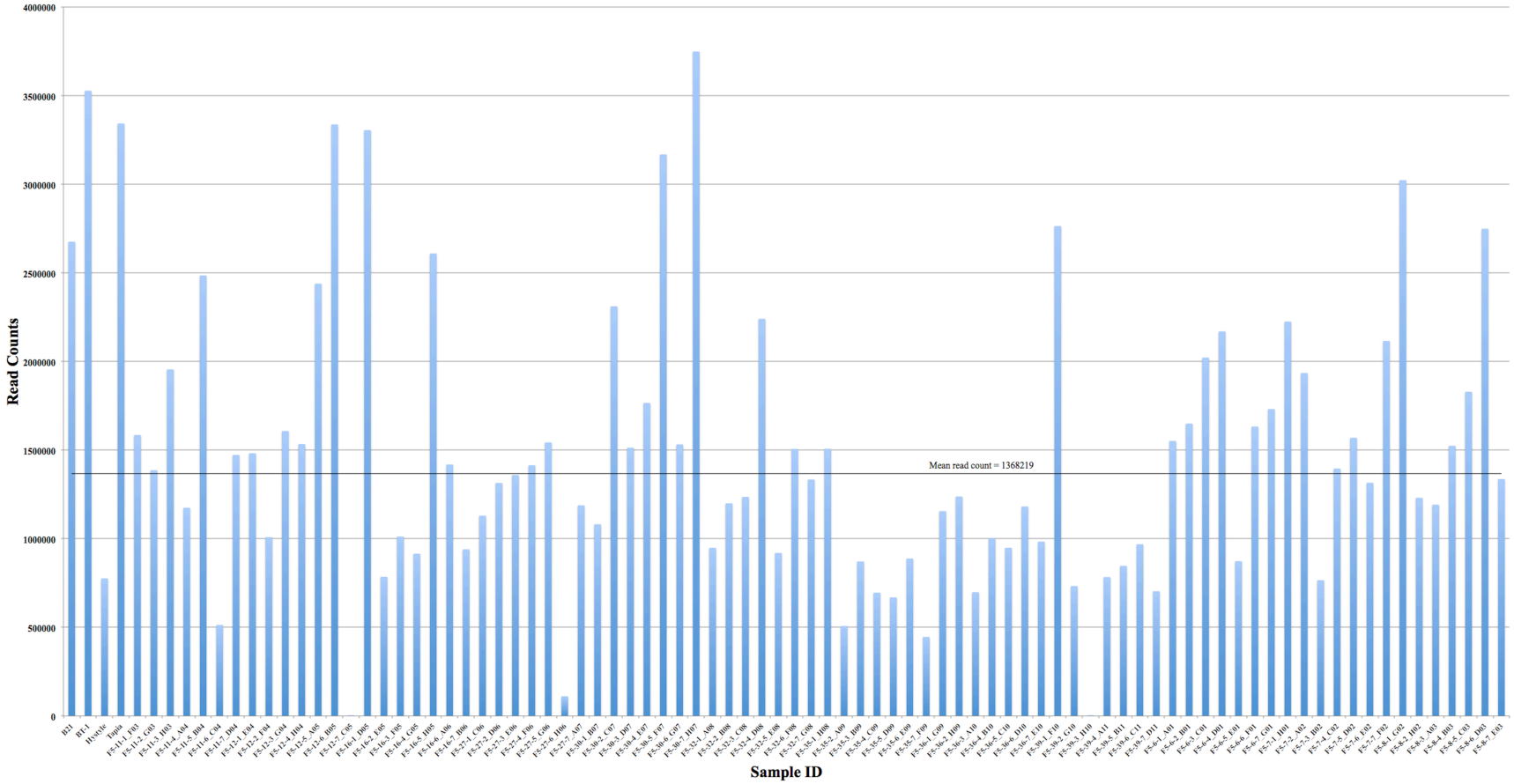
¶ Expected phenotypic ratio for F_2 population of 3-R [(including R(dSN)]:1-S; observed 149R:51S; χ^2 = 0.026, P = 0.87 (df = 1). Expected phenotypic ratio for F_2 population of 1-R: 2-R(dSN):1-S; observed 61-R: 88-R(dSN):51-S; χ^2 = 3.88, P = 0.14 (df = 2).

Expected phenotypic ratio for F_2 population of 3R [(including R(dSN)]:1S; observed 149-R:51-S; χ^2 = 0.48, P = 0.48 (df = 1). Expected phenotypic ratio for F_2 population of 1-R: 2-R(dSN):1-S; observed 22-R: 99-R(dSN):51-S; χ^2 = 12.53, P = 0.001 (df = 2).

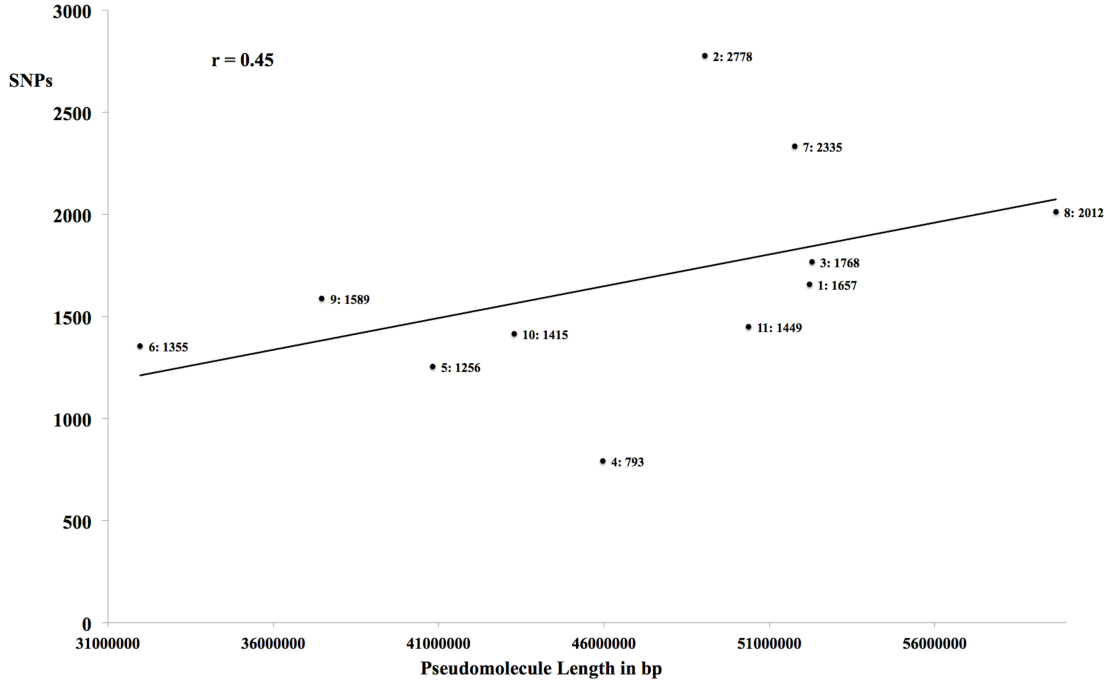
Appendix 4.7. Example fragment size distribution of GBS libraries made by digesting a 200 ng sample of *P. vulgaris* DNA with *Ape*KI or 500 ng of DNA with *Pst*I and analyzed by an Agilent BioAnalyzer 2100.



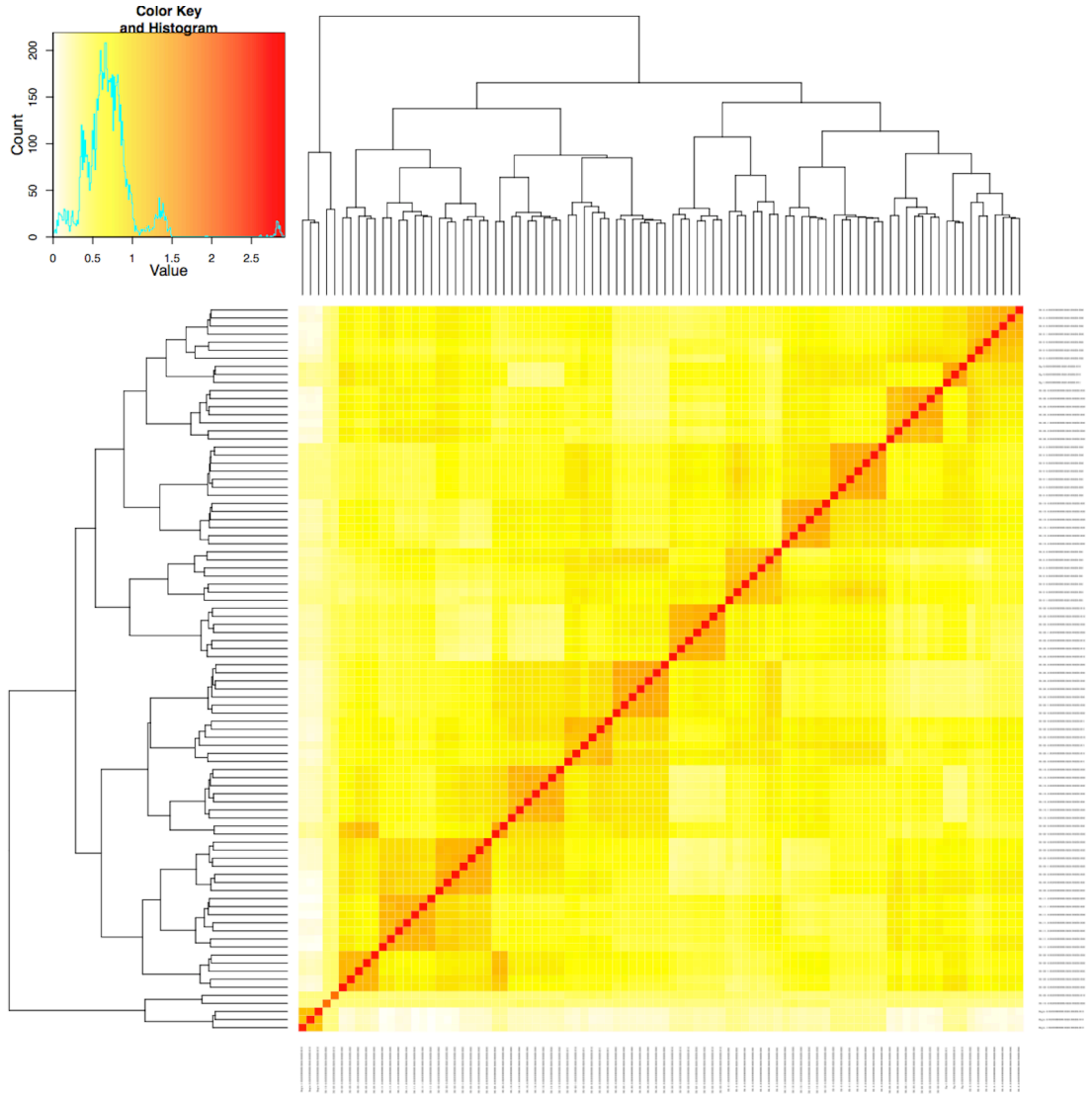
Appendix 4.8. Read counts per sample sequenced for the 96-plex *Ape*KI GBS library. The mean read count across all samples is displayed as grey horizontal line and was equal to 1,368,219 reads per sample.



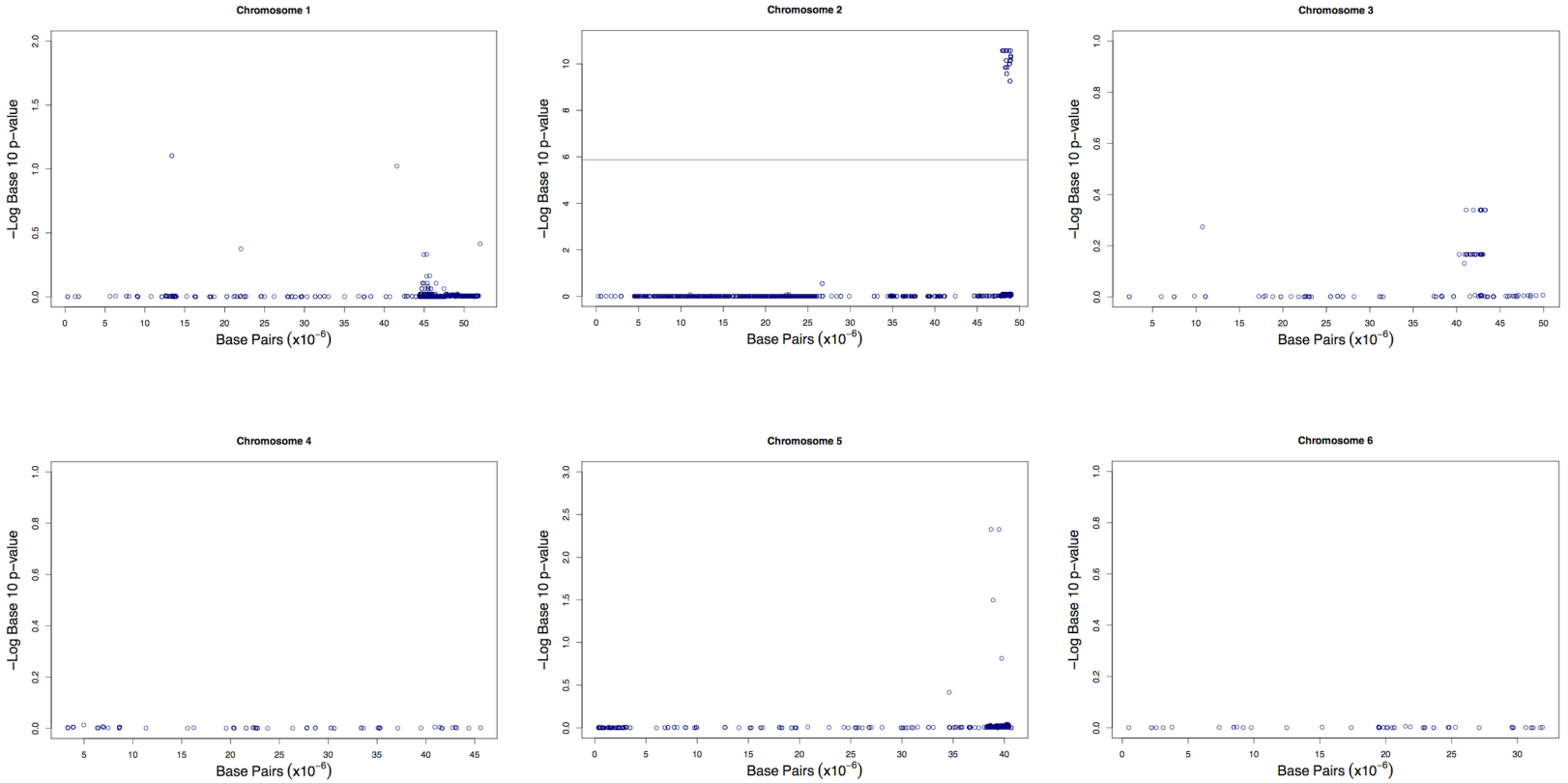
Appendix 4.9. The number of SNPs obtained per pseudomolecule (y-axis) plotted against the length of the pseudomolecule. Each pseudomolecule is labeled according the corresponding chromosome number, and is followed by the number of SNPs obtained. The coefficient of correlation between pseudomolecule length and number of SNPs obtained was r = 0.45.



Appendix 4.10. Heat map of kinship values. Although the labels are not visible at this scale, the heat map was included to verify and illustrate the structure of the germplasm that was genotyped.



Appendix 4.11. Results of the case-control genome-wide association study (GWAS) for *By-2* potyvirus presented by chromosome. The association of 7,530 single nucleotide polymorphisms (SNPs) (represented by open circles) with *By-2* resistance in 44 susceptible RILs (cases) and 42 resistant RILs (controls) is plotted as $-\log_{10}$ transformed *P* values (y-axis) against the physical positions in bp (x-axis) of each of the 11 chromosomes of the common bean genome. The green horizontal line indicates the $-\log_{10}$ *P* value threshold of the least statistically significant SNP as predicted by Bonferroni adjustment ($P \leq 1.3 \times 10^{-6}$).



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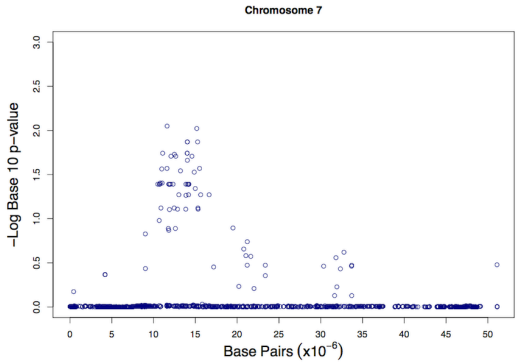
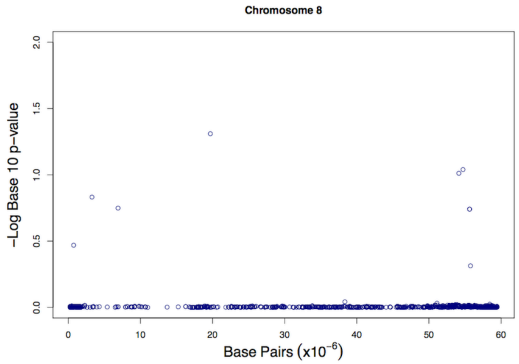
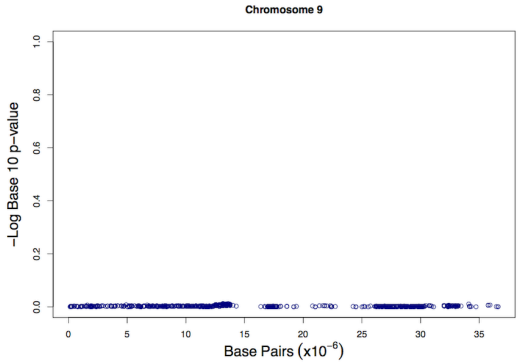
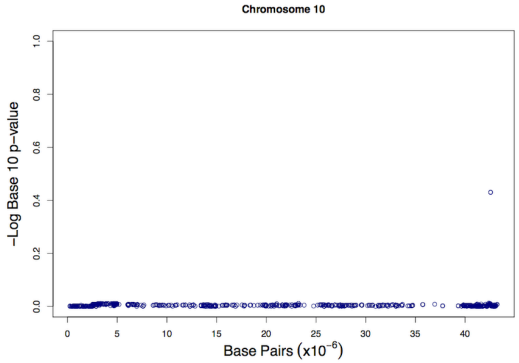
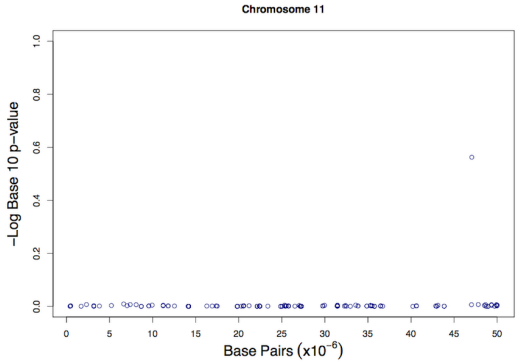
Appendix 4.11 Continued.











Appendix 4.12. Fourty-four SNPs significantly associated with *By-2* virus resistance, their *P*-values, minor allele frequency (maf), the number of observations (nobs), and their FDR adjusted *P*-values.

SNP	Chr	*P*-value	maf	nobs	FDR_Adjusted_*P*-values
S2_47991715	2	2.70E-11	0.478	90	1.45E-08
S2_47991735	2	2.70E-11	0.478	90	1.45E-08
S2_47991754	2	2.70E-11	0.478	90	1.45E-08
S2_48123417	2	2.70E-11	0.478	90	1.45E-08
S2_48391134	2	2.70E-11	0.478	90	1.45E-08
S2_48391165	2	2.70E-11	0.478	90	1.45E-08
S2_48469591	2	2.70E-11	0.478	90	1.45E-08
S2_48773661	2	2.70E-11	0.478	90	1.45E-08
S2_48964621	2	2.70E-11	0.478	90	1.45E-08
S2_48109422	2	2.70E-11	0.478	90	1.45E-08
S2_48151447	2	2.70E-11	0.478	90	1.45E-08
S2_48398135	2	2.70E-11	0.478	90	1.45E-08
S2_48469567	2	2.70E-11	0.478	90	1.45E-08
S2_48773673	2	2.70E-11	0.478	90	1.45E-08
S2_48946019	2	4.54E-11	0.483	90	1.87E-08
S2_48964445	2	4.54E-11	0.483	90	1.87E-08
S2_48964428	2	4.54E-11	0.483	90	1.87E-08
S2_48964453	2	4.54E-11	0.483	90	1.87E-08
S2_48965785	2	5.21E-11	0.483	90	1.87E-08
S2_48965798	2	5.21E-11	0.483	90	1.87E-08
S2_48965795	2	5.21E-11	0.483	90	1.87E-08
S2_48411199	2	7.13E-11	0.472	90	2.06E-08
S2_48458075	2	7.13E-11	0.472	90	2.06E-08
S2_48872722	2	7.13E-11	0.472	90	2.06E-08
S2_48902054	2	7.13E-11	0.472	90	2.06E-08
S2_48957206	2	7.13E-11	0.472	90	2.06E-08
S2_48875073	2	7.53E-11	0.489	90	2.10E-08
S2_48806075	2	9.96E-11	0.461	90	2.34E-08
S2_48806111	2	9.96E-11	0.461	90	2.34E-08
S2_48834859	2	9.96E-11	0.461	90	2.34E-08
S2_48834874	2	9.96E-11	0.461	90	2.34E-08
S2_48834890	2	9.96E-11	0.461	90	2.34E-08
S2_48553045	2	1.42E-10	0.478	90	3.00E-08
S2_48394072	2	1.42E-10	0.478	90	3.00E-08
S2_48553048	2	1.42E-10	0.478	90	3.00E-08
S2_48296773	2	1.43E-10	0.467	90	3.00E-08
S2_48487174	2	2.65E-10	0.456	90	5.00E-08
S2_48487184	2	2.65E-10	0.456	90	5.00E-08
S2_48487230	2	2.65E-10	0.456	90	5.00E-08
S2_48487175	2	2.65E-10	0.456	90	5.00E-08
S2_48872522	2	5.52E-10	0.450	90	9.45E-08
S2_48903379	2	5.52E-10	0.450	90	9.45E-08
S2_48872509	2	5.52E-10	0.450	90	9.45E-08
S2_48872510	2	5.52E-10	0.450	90	9.45E-08

Appendix 4.13 Case-Control genome-wide association study (GWAS) for delayed systemic necrosis The association of 7,530 single nucleotide polymorphisms (SNPs) (represented by open circles) is plotted as $-\log_{10}$ transformed *P* values on the y-axis against the physical positions of the 11 chromosomes of the common bean genome on the x-axis.